MEDICAL LIBRARY

SEP 8 1949

JOURNAL OF ANATOMY

ORIGINALLY THE JOURNAL OF ANATOMY AND PHYSIOLOGY

CONDUCTED ON BEHALF OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND BY

J. C. BRASH
J. D. BOYD
W. E. LE GROS CLARK
F. GOLDBY
W. J. HAMILTON
T. NICOL
J. M. YOFFEY
C. M. WEST (EDITOR)

VOLUME 83
PART 3
JULY 1949

Entered at New York Post Office as Second Class Matter

CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE, AND BENTLEY HOUSE, LONDON
AGENTS FOR U.S.A.
THE UNIVERSITY OF CHICAGO PRESS
CANADA AND INDIA: MACMILLAN

Price Twelve Shillings and Sixpence net

A NEW (ELEVENTH) EDITION OF

CUNNINGHAM'S MANUALS OF PRACTICAL ANATOMY

REVISED AND EDITED BY JAMES COUPER BRASH, M.C., M.D., D.Sc., F.R.C.S., F.R.S.E.

PROFESSOR OF ANATOMY IN THE UNIVERSITY OF EDINBURGH

In this, the first post-war edition of the standard dissecting manual adopted by so many medical schools throughout the English-speaking world, a serious attempt has been made to overcome the difficulties created by the increasing length and complexity of the curriculum. Recognising the burdens imposed on students by the need for the assimilation of a mass of anatomical detail in the primary stages, every effort has been made by the Editor to simplify practical teaching. It will be found that nothing of real importance has been discarded, all the features which helped to establish *Cunningham* in its unique position have been preserved, while due regard has been paid to the modern approach to the problems of medical education.

VOLUME 1: GENERAL INTRODUCTION. UPPER LIMB. LOWER LIMB
408 pages. 200 illustrations (112 in colour)

VOLUME 2: THORAX AND ABDOMEN
498 pages. 236 illustrations (129 in colour)

VOLUME 3. HEAD AND NECK. BRAIN
524 pages. 430 illustrations (116 in colour)

EACH VOLUME 21*s.* net

OXFORD UNIVERSITY PRESS

A Laboratory Guide to Vertebrate Dissection for Students of Anatomy

A. B. APPLETON

Copies are again available of this manual which aims at providing the guidance required by students in the actual dissection of the selected animals. Questions are included in the instructions, with answers given to the more difficult queries. 7*s.* 6*d.* net

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W.1

AGE CHANGES REVEALED BY CARBONYL REAGENTS IN TISSUE SECTIONS

By S. ALBERT and C. P. LEBLOND
Department of Anatomy, McGill University, Montreal, Canada

The histochemical reactions obtained with the two carbonyl reagents, fuchsin sulphurous acid (FSA) and 2,4-dinitrophenylhydrazine (DNPH), have been previously investigated in the organs of the rat (Albert & Leblond, 1946). A study of these reactions in mice tissues showed a widespread distribution similar to that found in the rat. Furthermore, the observations carried out in mice revealed that the intensity of the staining varied with the age of the animals. Thus, the carbonyl reactions present in sections of sex glands and accessory sex organs, such as seminal vesicles, uterus and mammary gland, usually became prominent at puberty, but tended to disappear in old animals.

This effect of ageing suggested that the staining intensity of many tissues varied with their physiological activity, and, therefore, the water-insoluble carbonyls (plasmalogens?) detected with FSA and DNPH may have more biological importance than hitherto believed.

MATERIAL AND METHODS

Age differences in the staining with FSA and DNPH were examined in mice of the high-cancer 'A' and low-cancer 'C57' strains at the ages of 1 day, 1, 2–3, 3–9, 11, 15 and 16 months. Four mice (two males and two females) of each strain were used per group. In the 15-month-old group, only 'A' mice were available. In addition, similar studies were carried out in four 8–10-month-old female mice of the high-cancer 'C3H' strain which bore mammary tumours.

A fairly complete study of the distribution of the reaction in almost all organs and tissues was carried out in several of the animals, but only the following tissues were systematically examined in all: adrenals, liver, kidney, heart, sex glands and accessory sex organs.

The technique employed in the staining of tissues with FSA was essentially that of the 'plasmal reaction' (Lison, 1936). This technique consisted of fixing the tissues in neutral formalin, washing in water, cutting frozen sections, and staining them with FSA after passage through a saturated solution of mercuric chloride.

Since erratic results and patchy staining were obtained in some of our earlier tests, experiments were carried out to determine the effects of various factors on the stainability of tissues with FSA. It was found that: (1) varying the duration of formalin fixation from 2 to 4, 24 or 48 hr. did not alter the intensity and patchiness of the stain; (2) no staining occurred if sections were cut after fixation and immediately taken through mercuric chloride and FSA without washing; (3) if after fixation the sections were allowed to stand in water in a shallow open dish overnight, intense and uniform staining resulted.

These observations suggested that oxidation by air may have played a role in the reaction. Indeed, if the washing of the sections was carried out in oxygen-free distilled water using completely filled, tightly stoppered bottles, very little or no reaction was observed with FSA. Conversely, the addition of 1–2 drops of 3% hydrogen peroxide (Merck, U.S.P.) per c.c. of tap water produced an intense and regular stain. Under these conditions, washing for $\frac{1}{2}$ hr. instead of 12–24 hr. was sufficient to ensure a good stain. Gérard (1935) had previously noted that the 'plasmal reaction' was not obtained after formalin fixation, unless subsequent oxidation was carried out.

It was concluded that staining after formalin fixation required some degree of oxidation using either the oxygen of the air or H_2O_2, each producing a regular and apparently identical staining pattern. It was also noted that sections, after such an oxidation, stained even if washing in mercuric chloride had been omitted. This treatment, however, frequently intensified the stain, but did not alter its distribution.

The oxidation provided by exposure to the air was adopted for routine work, since it was milder and, therefore, less likely to oxidize alcoholic to carbonyl groups in the sections. After a 48 hr. fixation in 10% neutral formalin and 24 hr. washing in running water, the tissue blocks were sectioned at 10μ on the freezing microtome. The sections were then washed in distilled water in shallow dishes for about 24 hr. prior to staining. During the night, they were kept in the cold room. The washed sections were placed in a saturated solution of mercuric chloride for 3 min., rinsed, left for 5 min. in FSA, washed in two solutions of sulphurous acid and mounted in glycerine-gelatine. A positive reaction was indicated by a purple colour. This colour was found to be very stable, since it has persisted for over a year in our material. Special care was taken to carry out the various operations simultaneously for all experimental groups, thus ensuring a uniform technical procedure.

The method used for staining sections with DNPH has been described previously (Albert & Leblond, 1946). The same precautions for fixation and washing as described above were taken in this procedure.

While most of the work was performed on formalin-fixed tissues, the FSA reaction was also examined in the fresh, unfixed tissues of two male hybrid albino mice and three female mice of the 'C3H' strain. These tissues were quickly removed from the animals, sectioned at 20–30μ on the freezing microtome, placed immediately in a saturated solution of mercuric chloride for 3 min. and stained with FSA for 5 min. Control slides were placed in FSA without pre-treatment with mercuric chloride.

In all cases the staining intensity of histological structures was rated in four different categories, namely, very intense, intense, moderate, and slight, and will be described below.

RESULTS OBTAINED WITH FIXED TISSUES

The general distribution of the FSA and DNPH reactions in the formalin-fixed tissues of 'A' and 'C57' mice was similar to that previously reported for the rat (Albert & Leblond, 1946). However, the staining intensity of most organs, especially kidney, adrenal cortex, corpus luteum and interstitial cells of the testis and ovary, was greater in mice than in rats. With both species there was a strict parallelism in the distribution of the FSA and DNPH reactions (Pl. 1, figs. 1, 2).

Endocrine glands

The staining of the *adrenal gland* varied markedly with age, although in all animals there was a definite staining of the cortex, but little or no staining of the medulla. In 1-day-old mice, the cortex stained intensely and rather uniformly throughout. There was no division of the cortex into the three zones normally found in older animals; the only suggestion of zoning being a less intense stain in a few superficial cells. At the age of 1 month, the adrenal cortex had become divided into three zones, of which the fascicular alone stained as intensely as did the whole cortex of the newborn. Both the glomerular and reticular zones were thin and stained slightly.

At the age of 3 months, the staining intensity of the fascicular zone reached its maximum. The thin glomerular and the thickened reticular zone stained only slightly, although more intensely than the medulla (Pl. 1, figs. 1, 2; Pl. 2, figs. 6, 7). In the 'A' females of this age group there was a band of cells distended by a large fatty vacuole (signet-ring cells) located between the reticular zone and the medulla. The fatty content of the large vacuole of these cells stained moderately, though it often fell out during the preparation of the sections (Pl. 2, fig. 6). The small ring of cytoplasm contained minute granules staining intensely. These signet-ring cells were not found in the adrenals of the 'C57' animals of corresponding age (Pl. 2, fig. 7). The reticular zone in this strain was uniformly thinner than in the 'A' animals, being hardly distinguishable in some cases. Furthermore, in the 'C57' animals, the cell cords of the fascicular zone were less regularly orientated; and the limit between the zones was not sharp, as typical fascicular cells extended into the reticular zone.

In the older age groups of both strains, the irregularity of the cell cords in the cortex became quite pronounced. The glomerular zone so lost its distinctness that it was unidentifiable in some of the oldest animals. In the fascicular zone, the cell cords stained less intensely and frequently lost their radial arrangement. Furthermore, a fairly intense reaction could usually be seen in the inner half of the fascicular zone, while its outer half reacted poorly (Pl. 3, fig. 17). In the oldest animals there was a breaking down of the inner fascicular cords into irregular, deeply staining masses and granules (Pl. 3, fig. 16). The reticular zone was also less regularly arranged than at 3 months, and its vascularization was increased, especially at its junction with the medulla. With age, the fatty globules described at the outer limit of the medulla in the 'A' strain disappeared. Finally, pigment was found in the reticular zone of mice of the later age groups in both sexes of both strains, especially in the 'C57'. These pigment masses appeared to be identical with those found in brown degeneration (Cramer & Horning, 1937; Lacassagne & Raynaud, 1937; Leblond & Nelson, 1937; Cook & Kennaway, 1940; Blaisdell, Gardner & Strong, 1941; Burrows, 1945; Tobin & Birnbaum, 1947).

In the *testis* of all but the oldest animals, the slight reaction found in the tubules contrasted with the good staining of the interstitial cells. These cells showed a moderate reaction as early as the first day of life (Pl. 4, fig. 21). The reaction was fairly intense at 1 and at 3 months (Pl. 4, fig. 22). In the 15-month-old mouse, however, there were only a few or even no cells giving a definite purple reaction in the interstitial spaces. The staining intensity of the Leydig cells, when any of them could

be identified, was much less than in the younger animals (Pl. 4, fig. 23). On the other hand, numerous irregular pigment masses were scattered throughout the interstitial spaces where the natural brown colour of the pigment contrasted with the light purple background in the FSA-stained slides.

In the *ovary* of all but the oldest animals, the slight reaction found in the contents of the Graafian follicles contrasted with the good staining of the thecal, interstitial and luteal cells. Thus in the follicles, the granulosa cells gave little or no reaction; the oocytes showed fine, discrete granules with a fairly intense colour, while the follicular fluid did not react at all. The thecal cells gave an intense colour, but in places their reaction was moderate (Pl. 2, figs. 8, 9). The interstitial cells showed a moderate reaction at 1 month, a very intense one at 3 months (Pl. 2, figs. 8, 9), and a decreased reaction at the later age intervals, especially at 15 months, when they either stained slightly or not at all (Pl. 3, figs. 13, 15). The atretic follicles sometimes showed a few intensely staining patches corresponding to groups of lipid-laden cells. There was a wide range in the staining intensity of corpora lutea. In the 3-month-old animal of the 'C57' strain, for instance, intensely staining corpora lutea were seen next to moderately staining ones (Pl. 2, fig. 9). In the oldest animals, corpora lutea were seen which stained very little or not at all (Pl. 3, fig. 15).

In general, as the animals grew older, the following changes became apparent, starting at 8 months. Whole groups of interstitial cells and, to a smaller extent, thecal cells, more or less completely lost their ability to stain with FSA (Pl. 3, figs. 13, 15). Next to these cells, masses of pigment became visible and gradually increased with age (Pl. 3, figs. 12, 14). Finally, large unstained areas with a glassy appearance were seen. These were considered to be old corpora lutea (Pl. 3, fig. 15). These structures sometimes showed small groups of irregularly staining globules. One of the 15-month-old 'A' mice showed a completely atrophic, non-staining ovary.

Accessory sex organs

In the male, a detailed study of the *seminal vesicles* was made at various ages. An intense reaction was present in the vacuoles of the cell apex, as described in the rat (Albert & Leblond, 1946). This apical reaction was slight to moderate at 1 month, most intense at 3 months (that is to say, when males reach sexual maturity), moderate at 11 months and very slight at 15 months. While the cell apex stained intensely, the reaction in the base was only moderate. A slight reaction was present in the smooth muscle and a slight to moderate reaction in the lamina propria, probably due to the presence of elastic fibres. The secretion present in the lumen did not react.

In the female, the *uterus* showed a slight reaction in the smooth muscle and a fairly intense reaction in the epithelial cells, but only in sexually mature animals. Thus the 3-month-old 'C57' females showed a definite reaction in the epithelium and glands (Pl. 2, fig. 11), the apical and particularly the basal regions of the cells containing minute, brightly staining granules. In contrast, the uteri of the more slowly maturing 'A' females showed little or no reaction at 3 months (Pl. 2, fig. 10). Older 'A' strain females showed stained granules in the epithelium. It may be noted that scattered, intensely staining cells could be found in the mesometrium (Pl. 2, fig. 11). Finally,

brownish pigment was found to accumulate in the lamina propria and myometrium of the older animals.

The *mammary glands* showed irregularly occurring reactions in the cells of the epithelium, apparently located on fatty inclusions (Pl. 1, fig. 3). Brightly staining fat globules were found free in the lumen as described by Dempsey, Bunting & Wislocki (1947).

A series of females of the high-cancer 'C3H' strain were examined with regard to the FSA reaction of cancerous and non-cancerous mammary tissue. While a moderate to intense reaction was observed in the non-cancerous mammary epithelium (Pl. 1, fig. 3), little or no reaction was present in a cystadenocarcinoma and in three adenocarcinomata (Pl. 1, fig. 4).

Other organs

The reactions in the other organs were on the whole quite comparable with, although more intense than, those described in the rat (Albert & Leblond, 1946). Thus a definite reaction—slight to moderate—was present in the lymphatic organs of the mouse.

The *liver* of animals of all ages showed a fairly intense staining, restricted to the cell cytoplasm. The most pronounced reaction was observed in the youngest animals with a tendency towards a slow decrease with ageing. Individual variations were considerable in this organ. Deep-staining globules were occasionally seen in the Kupffer cells, a fact in keeping with the frequent occurrence of intense reactions in cells of the reticulo-endothelial system in all locations (Albert & Leblond, 1946).

In the *kidney* of adult animals, the reaction was maximal in the proximal convoluted tubules, moderate or fairly intense in the outer medulla (distal convoluted tubules and Henle's loops), and absent in the collecting tubules, while the glomeruli showed little or no reaction. The structures showing the greatest variation with age were the proximal convoluted tubules. In the newborn, the proximal convoluted tubules developing in the outer part of the organ did not react. In the middle region, however, where a few differentiated proximal convoluted tubules were apparent, slight to moderate reactions were found. In the innermost part of the organ, where only collecting tubules are located, no staining occurred (Pl. 4, fig. 18). An intense uniform staining of the proximal convoluted tubules in the cortex was observed in 1- and 3-month-old animals (Pl. 4, fig. 19). As the animals aged, some of the proximal convoluted tubules did not take up the stain, and eventually most of them remained unstained (Pl. 4, fig. 20). The occurrence of scattered, irregular globules in the collecting tubules may also be observed, especially in the older age groups.

In *cardiac muscle* the reaction was found to be moderate in the newborn animals, but fairly intense in all other groups.

RESULTS OBTAINED WITH FRESH (UNFIXED) TISSUES

Fresh tissues taken to FSA without passing through mercuric chloride did not stain at all. On the other hand, pre-treatment with the mercuric salt resulted in an intense purple staining in most organs. The reactions thus obtained were usually similar to those observed in formalin-fixed tissues. Thus, the ovary showed a fairly intense reaction in the corpora lutea, thecal and interstitial cells, but not in the granulosa.

The kidney showed a fairly intense reaction in most proximal convoluted tubules, and little or no reaction in the glomeruli. The outer medulla reacted in a regular fashion since all tubes showed a fairly intense staining.

Although Hayes (1947) noted that in most tissues of the rat the staining was the same before and after fixation, he emphasized the difference in the reactions of adrenal cortex and liver before and after fixation. In the mouse, however, the adrenal cortex showed the same type of reaction in the fresh state (Pl. 1, fig. 5) as after fixation (Pl. 1, fig. 1; Pl. 2, figs. 6, 7). The medulla, on the other hand, reacted in the fresh state (Pl. 1, fig. 5) but not after fixation (Pl. 1, fig. 1; Pl. 2, figs. 6, 7). The case of the liver was quite different, since only a very slight or no reaction was observed in the fresh state, while a definite staining occurred after formalin fixation.

DISCUSSION

Chemical nature of the tissue compounds reacting with FSA and DNPH

It must first be emphasized that no staining with FSA or DNPH was observed in any fresh mouse tissue without pre-treatment with mercuric chloride. Thus, there was no significant amount of free reactive carbonyls in any organ.

The intense staining observed after passage of unfixed sections through mercuric chloride—a reagent known to free carbonyls from acetal linkage—indicated that the carbonyls stained with FSA or DNPH must have been bound in this type of linkage.

Furthermore, the reacting carbonyls or their precursors could readily be extracted from the sections by fat solvents, but not by water, a fact previously noted by Hayes (1947). The lipidic nature of the reacting material suggested by this observation was confirmed by the frequent localization of the purple stain on fat droplets.

Two types of carbonyl lipids are known to exist in tissues: the ketosteroids, in which the carbonyl radical is a free ketonic group, and the plasmalogens, which are phospholipid derivatives with an aldehydic carbonyl combined in acetal linkage. From the outset, it was apparent that the plasmalogen aldehydes behaved like the carbonyls detected in sections with FSA. However, the claim of the Harvard school that FSA may be used for the histochemicaldetection of steroids (Dempsey, Bunting & Wislocki, 1947) makes it necessary to re-examine the possibility that ketosteroids play a role in this staining.

If ketosteroids were the reacting material, fresh tissues would stain without mercuric chloride treatment. Furthermore, the rapid development of the reaction with FSA after action of the mercuric salt indicated that the freed carbonyls must have been aldehydic rather than ketonic, since the reactivity of aldehydes was found to be considerably greater than that of most ketones as judged by sulphite addition (Petrenko-Kritschenko, 1905) or semi-carbazide formation (Conant & Bartlett, 1932). Finally, *in vitro* experiments (Oster & Oster, 1946; Albert & Leblond, 1946; Hayes, 1947; Boscott, Mandl, Danielli & Shoppee, 1948) showed that tissue extracts treated with mercuric chloride reacted with FSA in an intense fashion, while many ketones and especially ketonic steroids did not. Oster & Oster (1946) noted that some short-chain ketones reacted with FSA, namely, acetone, methyl ethyl ketone, methyl iso-butyl ketone; but they developed a red colour quite different from the characteristic purple colour obtained with aldehydes. Furthermore, the hormonally

active ketosteroids, testosterone propionate, methyl testosterone, dehydroandrosterone acetate, androstenedione and pregnenolone, gave no reaction with FSA. It was, therefore, concluded that ketosteroids were not responsible for the staining of tissues with this reagent.

The only other lipidic carbonyls known to exist in tissues were the aldehydes of the higher fatty acids, especially palmitaldehyde and stearaldehyde, which have been isolated from muscle and brain by splitting the acetal-phosphatide plasmalogen (Feulgen, Imhauser & Behrens, 1929; Anchel & Waelsch, 1942). Since the properties of plasmalogen satisfactorily accounted for those of the material detected in fresh sections with FSA, it was concluded that this material was plasmalogen. Recently, it was suggested that there existed in the body an acetal lipid lacking phosphorus and the colamine base (Newman, 1944) as well as other combined lipid aldehydes (Oster & Schlossman, 1946); but no more than presumptive evidence of their presence was given.

Incidentally, cellular components 50–200 mμ in size obtained by ultra-centrifugation of tissues gave a positive reaction with FSA. Furthermore, half the material in these particles was shown by phosphorus and nitrogen analysis to be phospholipidic (Claude, 1940, 1941). These results were thus in agreement with the opinion that the FSA-staining material in tissues consists of acetal phosphatides.

While this conclusion was based on results obtained with fresh tissues, it did not necessarily apply to fixed tissues. Thus, Hayes (1947) assumed that plasmalogen was detected by FSA after mercuric chloride treatment but only when fresh tissues were used. The present experiments, however, showed that, at least in the mouse, the localization of the reactions in most organs (ovary, adrenal cortex, kidney, etc.) was identical under fresh and fixed conditions. Therefore, the purple staining observed in fixed sections must have been derived from the same source as in fresh tissues. Plasmalogen thus appeared as being also the cause of the reactions observed in fixed tissues.

The staining of the fresh adrenal medulla (Pl. 1, fig. 5) contrasted with the absence of a reaction after fixation (Pl. 1, fig. 1; Pl. 2, figs. 6, 7). Probably the reacting aldehyde was extracted during the technical procedure and must therefore have been more soluble than the material in other organs and tissues. Whether the bound aldehyde present in the adrenal medulla differed from plasmalogen could only be surmised.

The liver was the only organ in which no reaction was present in the fresh condition, but some appeared after fixation. Hayes (1947) assumed that such behaviour indicated the formation of oxidation products independent of plasmalogen. However, we were able to demonstrate the presence in the liver of a plasmalogen type of compound, since a chloroform extract of this organ reacted with FSA after mercuric chloride treatment (unpublished experiments). Thus, it appeared more likely that some inhibitory mechanism prevented the reaction of plasmalogen in fresh liver sections.

The conclusion that plasmalogen was the source of the carbonyls detected in fixed tissues by FSA and DNPH must be reconciled with the result of the experiments on formalin fixation. In describing the technique, it was pointed out that the reaction was inhibited by formalin fixation, but could be reconstituted by oxidation. The possibility existed that during the process of formalin fixation, the chemistry of which

is poorly understood, some of the acetal phosphatides were slowly disrupted and the released fatty aldehydes were partly reduced. During subsequent oxidation the aldehydes would be reconstituted into an active form. That such a process occurred to some extent was suggested by the fact that reactions could take place without mercuric chloride in fixed sections, but were usually intensified by this reagent, while their localization was not altered.

It was thus concluded that, with a few possible exceptions, the reactions obtained in fresh and formalin-fixed tissues of the mouse with FSA and DNPH were due to lipid aldehydes bound as plasmalogen.

Influence of age on FSA and DNPH reactions

It is apparent that the staining of most organs with FSA and DNPH was markedly influenced by the age of the animals. In most organs, there was a gradual overall decrease of the staining intensity with advancing age. The results were most striking in the gonads and accessory sex organs. Here the reactions were slight or absent at birth, increased rapidly to reach a maximum at sexual maturity, remaining definite for a number of months during the active sexual life, and then decreasing with advancing age to become slight or to disappear in senility. Thus, for instance, in the epithelium of the seminal vesicle, the reaction became intense at puberty and disappeared in senile animals. This cycle may be compared to what occurred after castration and substitution therapy, since castration almost completely suppressed the reactions of seminal vesicles and prostate, while testosterone treatment restored them (Albert & Leblond, 1946). Therefore, the staining intensity of the seminal vesicle was dependent on its functional activity.

Similarly, there was an obvious parallel in the testis between the function of the interstitial cells and their staining reaction, which was moderate at birth (Pl. 4, fig. 21), most intense at puberty (Pl. 4, fig. 22) and slight in old age (Pl. 4, fig. 23). A relation between the depth of the staining and physiological activity was also encountered in the ovary (Pl. 2, figs. 8, 9; Pl. 3, figs. 12–15), in the kidney (Pl. 4, figs. 18–20), and to a lesser extent in the adrenal. A similar correlation between increased FSA reactions and functional activity was postulated by Tonutti (1941) to explain the intensified reactions found in the adrenal cortex of animals treated with diphtheria toxin and corticotrophin. In the skin, only the deeper, more active layers of the epidermis stained with FSA (Voss, 1941).

In unpublished experiments we found that hypophysectomy produced some decrease in the intensity of the staining reaction in the adrenals, gonads and accessory sex organs. The decrease and shift in the FSA reaction of the adrenals produced by this operation has already been examined by Deane & Greep (1946). Since removal of the hypophysis reduces the elaboration and release of steroid hormones by adrenals and gonads, these results supplied another example where staining varied as the physiological activity.

From these numerous examples it may be concluded that in many tissues the intensity of the reactions paralleled the functional state and, therefore, the amount of aldehydes combined in acetal bondage increased as the organs became actively functioning.

Since the amount of a substance present in an organ is the result of a balance

between the quantity elaborated or incorporated into the organ, and the quantity used up or excreted, the presence of a greater amount of bound aldehydes in active than in resting tissues indicated a predominance of accumulation over elimination. It is not known whether this accumulation is the result of the binding of aldehydes for their detoxication as suggested by Feulgen or is rather a preparatory step for an enzymatic release of aldehydes to be used in cellular reactions.

While the biological role of these bound aldehydes is unknown, a few preliminary conclusions may be drawn: The presence of a constant intensity of staining as in the interstitial cells of the testis should indicate a constant metabolism of plasmalogen and presumably a steady rate of secretion by all the cells, while a variable staining as in the interstitial cells of the ovary may be referable to a cyclic secretory activity.

The decrease in carbonyl staining occurring in ageing animals was frequently accompanied by an increase in the amount of brownish 'wear and tear' pigment, especially in the steroid-secreting cells (adrenal, cortex, interstitial cells of testis, thecal and interstitial cells of ovary, corpus luteum), where large, irregular cells laden with brown pigment often appeared and gradually increased in amount (Pl. 3, figs. 12–15). A similar pigmentation has been described in the interstitial spaces of the testis of old mice by Hooker and Pfeiffer (1942). The steps of this pigment degeneration seemed to be as follows. At first, minute brown masses appeared in the cytoplasm of some of the cells. Later, these cells were phagocytosed by reticulo-endothelial cells, which then made up the clumps of irregular, pigment-laden masses described above. At any rate, this pigment degeneration of the steroid-secreting cells always occurred as their content in bound aldehyde decreased and their secretory role was reduced or terminated.

There is an exception to the rule that the staining with FSA and DNPH indicates the functional activity of steroid-secreting and other organs. A marked staining was observed in a few cells with signs of fatty degeneration. Thus, intensely staining fatty globules could be observed in degenerating follicles and old corpora lutea of the ovary, in the reticular zone of the adrenal, occasionally in the collecting tubules of the kidney and fairly often in the cells of the reticulo-endothelial system. In all these cases, the presence of the material in large, distended fat globules pointing to fatty degeneration cannot be taken as indicating functional activity, but rather a passive storage of the material.

More experience may be needed to assess accurately the value of FSA staining as an index of function. However, since it is most difficult to estimate the secretory ability of cells such as the interstitial cells of testis and ovary on ordinary histological slides, the technique described above may provide a new instrument.

SUMMARY

The carbonyl reagents (fuchsin sulphurous acid and dinitrophenylhydrazine) were used as histochemical stains in a study of the tissues of 'A' and 'C57' mice taken at birth and at 1, 2–3, 8–9, 11, 15 and 16 months of age. The carbonyls detected in fresh and fixed tissues appear to be aldehydic lipids held in acetal linkage, presumably as 'plasmalogen'.

The histochemical reactions obtained with the two stains have the same distribution. They are present in almost all organs and tissues, but they are most intense

in the steroid-secreting cells: adrenal cortex, interstitial cells of testis and ovary, corpus luteum.

The staining intensity of kidney, endocrine glands, sex glands and accessory sex organs increases until puberty, is maintained during sexual maturity and subsequently decreases with increasing age. Frequently, the decrease or disappearance of the carbonyl reactions is accompanied by pigment degeneration, especially in the steroid-secreting cells.

The parallel existing between the staining with carbonyl reagents and the physiological level of activity suggests the use of these as tests of function. Some caution is, however, necessary in the interpretation of the results.

This work was carried out with the help of grants from the Biological Stain Commission and the Donner Foundation. The authors wish to acknowledge the help of the Misses C. E. Stevens and W. F. Storey. The mice were a gift from Dr C. Fraser.

REFERENCES

Albert, S. & Leblond, C. P. (1946). The distribution of the Feulgen and 2,4-dinitrophenylhydrazine reactions in normal, castrated, adrenalectomized and hormonally treated rats. *Endocrinology*, **39**, 386–400.

Anchel, N. & Waelsch, H. (1942). The higher fatty aldehydes. Isolation from small amounts of tissue with acidic carbonyl reagents. *J. biol. Chem.* **145**, 605.

Blaisdell, J. S., Gardner, W. A. & Strong, L. C. (1941). Adrenal glands of mice from strains with different susceptibilities to mammary carcinoma. *Cancer Res.* **1**, 283–289.

Boscott, R. J., Mandl, A. M., Danielli, J. F. & Shoppee, C. W. (1948). Cytochemical demonstration of ketosteroids. *Nature, Lond.*, **162**, 572.

Burrows, H. (1945). The occurrence of perineal hernias in untreated male mice of the C57 black strain. *Yale J. Biol. Med.* **17**, 568–570.

Claude, A. (1940). Particulate components of normal and tumor cells. *Science*, **91**, 77–78.

Claude, A. (1941). Particulate components of cytoplasm. *Cold Spr. Harb. Symp. Quant. Biol.* **9**, 263–270.

Conant, J. B. & Bartlett, P. D. (1932). A quantitative study of semicarbazone formation. *J. Amer. chem. Soc.* **54**, pt. 2, pp. 2881–2899.

Cook, J. W. & Kennaway, E. L. (1940). Chemical components as carcinogenic agents. Second supplementary report: Literature of 1938 and 1939. *Amer. J. Cancer*, **39**, 521.

Cramer, W. & Horning, E. S. (1937). Adrenal changes associated with oestrin administration and mammary cancer. *J. Path. Bact.* **44**, 633.

Deane, H. W. & Greep, R. O. (1946). A morphological and histochemical study of the rat's adrenal cortex after hypophysectomy, with comments on the liver. *Amer. J. Anat.* **79**, 117–146.

Dempsey, E. W., Bunting, H. & Wislocki, G. B. (1947). Observations on the chemical cytology of the mammary gland. *Amer. J. Anat.* **81**, 309–342.

Feulgen, R., Imhauser, K. & Behrens, M. (1929). Zur Kenntnis des Plasmalogens. 1. Mitt.: Eigenschaften des Plasmalogens, Darstellung und Natur des Plasmals. *Hoppe-Seyl. Z.* **180**, 161.

Gérard, P. (1935). Sur la réaction plasmale. *Bull. Histol. Tech. micr.* **12**, 274.

Hayes, E. R. (1947). A histochemical study of carbonyl lipids. Dissertation, Ohio State University, Columbus, Ohio.

Hooker, C. W. & Pfeiffer, C. A. (1942). The morphology and development of testicular tumors in mice of the A strain receiving oestrogens. *Cancer Res.* **2**, 759–769.

Lacassagne, A. & Raynaud, A. (1937). A propos l'action de l'oestrone sur la surrénale; les symplasmes phagocytaires chez la souris. *C.R. Soc. Biol., Paris*, **124**, 1183–1186.

Leblond, C. P. & Nelson, W. O. (1937). Étude histologique des organes de la souris sans hypophyse *Bull. Histol. Tech. micr.* **14**, 181–204.

Lison, L. (1936). *Traité d'histochimie animale; méthodes et problèmes.* Paris: Gauthier-Villars.

Newman, W. T. (1944). Studies on the acetal phospholipids. Thesis, University of Rochester.

Oster, K. A. & Oster, J. G. (1946). The specificity of sex hormones on the tissue aldehyde shift in the rat kidney and of fuchsin sulfurous acid reagent on aldehydes. *J. Pharmacol.* **87**, 306–312.

Oster, K. A. & Schlossman, N. C. (1946). Histochemical demonstration of amine oxidase in the kidney. *J. cell. comp. Physiol.* **20**, 373–378.

Petrenko-Kritschenko, P. (1905). Zur Charakteristik der Keton- und Aldehydreactionen. *Liebigs Ann.* **341**, 151–171.

Tobin, C. E. & Birnbaum, J. P. (1947). Some factors influencing brown degeneration of the adrenal gland in the Swiss albino mouse. *Arch. Path. Lab. Med.* **44**, 269–281.

Tonutti, E. (1941). Histochemische Befunde an der Diptherienebenniere mittels der Plasmalreaktion. *Klin. Wschr.* **20**, 1196–1198.

Voss, H. (1941). Histotopochemische Untersuchungen über das Verhalten der menschlichen Haut zur Plasmalreaktion. *Dtsch. med. Wschr.* **67**, 127.

EXPLANATION OF PLATES

Plate 1

Fig. 1. Adrenal of 3-month-old 'A' mouse. FSA stain. The grey to black colour represents the purple seen in tissue sections. The black spots in the medulla are usually due to blood in the blood vessels. The glomerular zone stains slightly, the fascicular zone intensely, the wide reticular zone slightly, and the medullary zone not at all. The darkly staining circles at the reticulo-medullary junction represent the signet-ring cells described in the text. Photograph taken with daylight filter on panchromatic plate. ×40.

Fig. 2. Same organ as in fig. 1, stained with DNPH and showing the same pattern of staining as with FSA. Photograph taken with a purple filter on panchromatic plate. ×40.

Fig. 3. FSA reactions in normal mammary gland of a 9-month-old female 'C3H' mouse. The cells of the alveoli contain intensely staining, irregular droplets, while the ducts and stroma stain slightly or not at all. Photograph taken with a daylight filter on contrast plate. ×40.

Fig. 4. Adenocarcinoma from a 9-month-old female 'C3H' mouse. The epithelial and stromal elements stain slightly or not at all. Photograph taken as in Fig. 3. ×40.

Fig. 5. Adrenal gland of a mouse sectioned while fresh, and stained with FSA after mercuric chloride treatment. Intense reaction in both cortex and medulla. Compare with sections of fixed adrenal glands (figs. 1, 6 and 7). ×94.

Plate 2

Comparison of FSA reactions in organs of a 3-month-old 'A' female mouse (left) and a 3-month-old 'C57' female mouse (right). Photographs taken with daylight filter on panchromatic plates. The black colour represents the purple reaction. ×40.

Fig. 6. 'A' adrenal. The glomerular zone stains slightly, the fascicular zone intensely, the wide reticular zone slightly, and the medullary zone not at all. The darkly staining circles at the reticulo-medullary junction represent the signet-ring cells described in the text.

Fig. 7. 'C57' adrenal. The glomerular zone stains slightly, the fasicular zone intensely, the narrow reticular zone slightly, and the medullary zone not at all. Note the absence of the darkly staining signet-ring cells at the reticulo-medullary junction, and the narrowing of the reticular zone compared with fig. 6.

Fig. 8. 'A' ovary. The interstitial and thecal cells stain intensely, the granulosa cells slightly or not at all. Corpora lutea are absent.

Fig. 9. 'C57' ovary. The interstitial and thecal cells stain moderately to intensely, the corpora lutea slightly to intensely, and the granulosa cells slightly or not at all. The presence of corpora lutea indicated an early maturity in this strain.

Fig. 10. 'A' uterus. The immature muscularis and epithelium stain slightly or not at all.

Fig. 11. 'C57' uterus. The well-developed muscularis stains slightly or not at all, the basal portions of the epithelial cells intensely, and the apex only slightly. A concentration of positively staining material may be seen in the mesometrium.

Plate 3

FSA reactions in senile animals. Figs. 12 and 14 were taken with a daylight filter on contrast plates and show in black both the purple staining reaction and the brown pigment. Figs. 13 and 15 were taken with a green filter on panchromatic plates to show in black the purple reaction only. It is, therefore, possible to deduce the location of the brown pigment, which is the material appearing in black in 12 and 14, but not in 13 and 15. ×40.

Fig. 12. 8-month-old 'C57' mouse ovary. The deposition of the non-staining but light absorbing brown pigment in the interstitial cells can be determined by the presence of black interstitial masses visible in this and not in the next figure.

Fig. 13. Same tissue as fig. 12 showing in black the FSA stain only. Only one corpus luteum and rare groups of thecal and interstitial cells stain.

Fig. 14. 16-month-old 'A' mouse ovary. The brown pigment can be recognized by the dark spots in this figure.

Fig. 15. Same tissue as fig. 14 showing only FSA stain. The entire ovary, with the exception of the cells lining an ovarian cyst, stains slightly or not at all. The hyalinized corpora lutea do not stain at all.

Fig. 16. 16-month-old female 'A' mouse adrenal. The glomerular zone stains moderately, the outer half of the fascicular zone moderately, and the inner half intensely. Abundant aggregations of darkly staining cells extend from the inner fascicular zone into the indistinct reticular zone. A small amount of pigment is also present.

Fig. 17. 16-month-old female 'A' mouse adrenal. The glomerular zone stains slightly, the outer half of the fascicular zone slightly to moderately, and the inner half intensely; the reticular zone slightly, and the medulla not at all. Throughout the reticular zone are scattered intensely staining aggregations of cells.

PLATE 4

Variations of FSA reaction in kidney (left) and testis (right) with age. The black colour represents the purple seen in the tissues. Photographs taken with a daylight filter on contrast plates. ×40.

Fig. 18. 1-day-old mouse kidney. A few differentiated proximal tubules in the middle region stain slightly. No stain anywhere else.

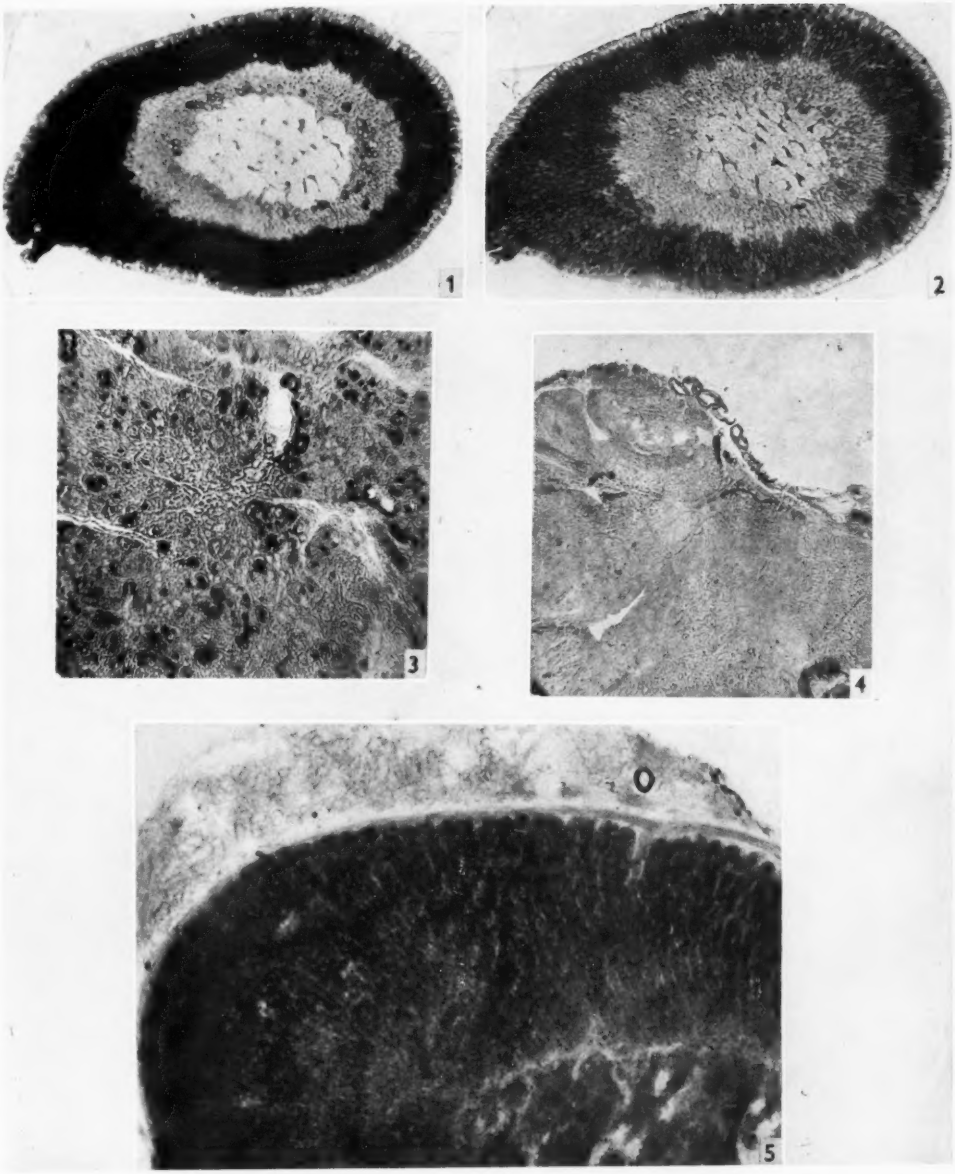
Fig. 19. 3-month-old mouse kidney. Fairly intense reaction in the proximal convoluted tubules.

Fig. 20. 16-month-old mouse kidney. Slight or no staining throughout the organ. This kidney showed the least intense reaction of all those examined.

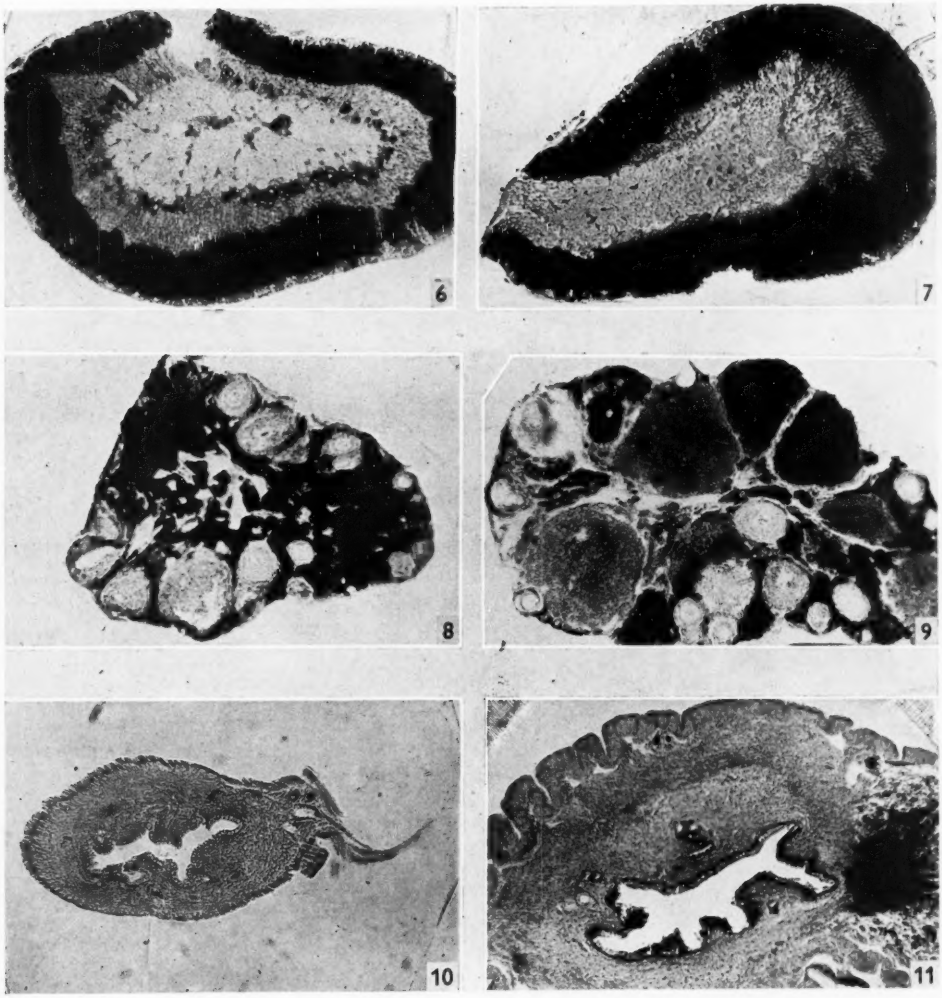
Fig. 21. 1-day-old mouse testis. The interstitial cells stain fairly intensely and the poorly developed spermatogenic tubules slightly or not at all.

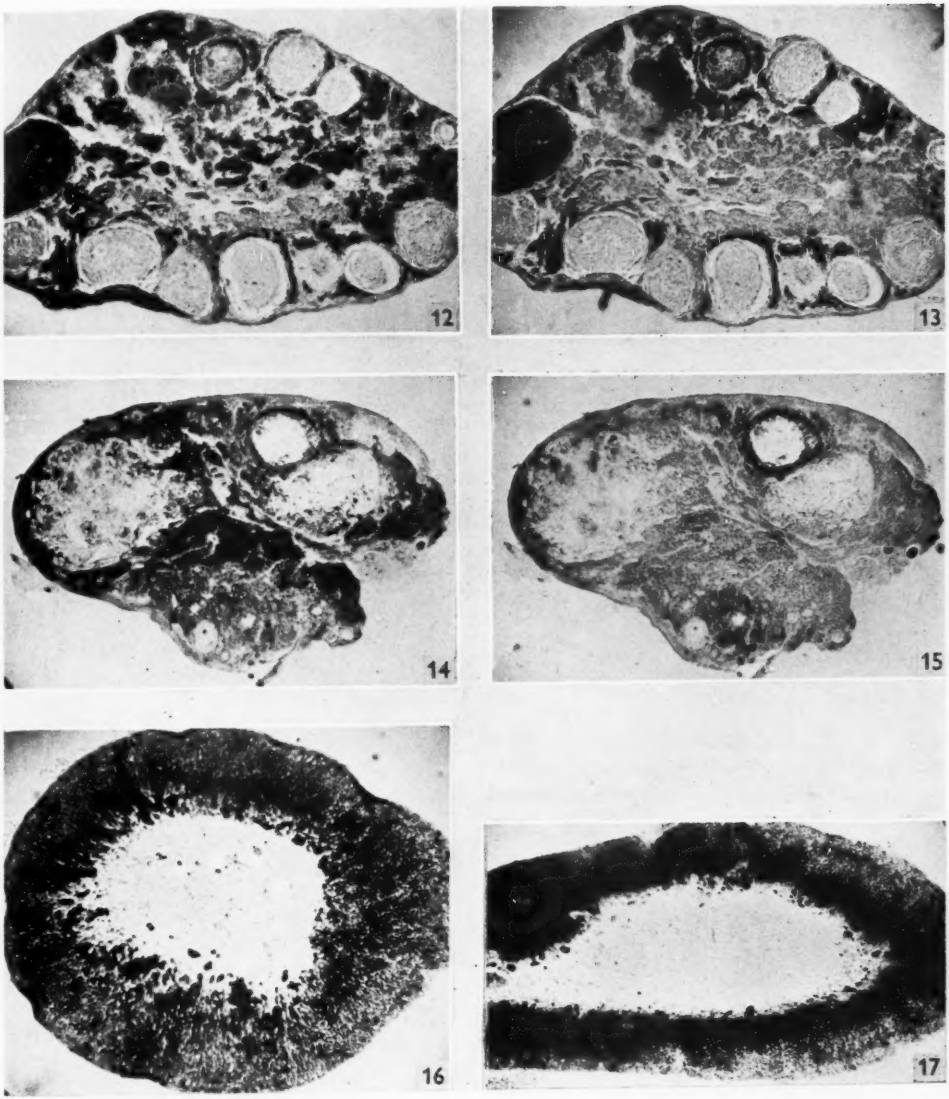
Fig. 22. 3-month-old mouse testis. The interstitial cells stain intensely and the spermatogenic tubules slightly or not at all, with a few dark staining granules about the heads of the spermatids.

Fig. 23. 15-month-old mouse testis. The atrophied cells of the interstitial spaces and the spermatogenic tubules stain slightly or not at all.

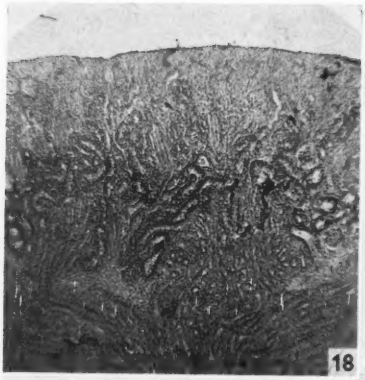


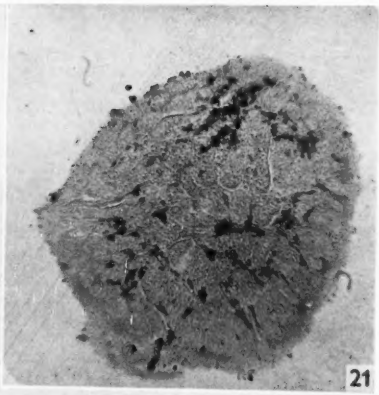
ALBERT AND LEBLOND—AGE CHANGES REVEALED BY CARBONYL REAGENTS

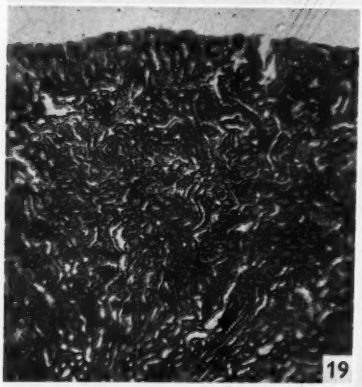

6
7
8
9
10
11

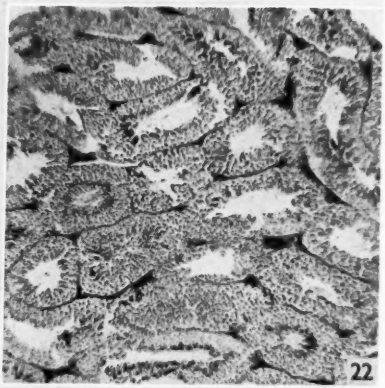


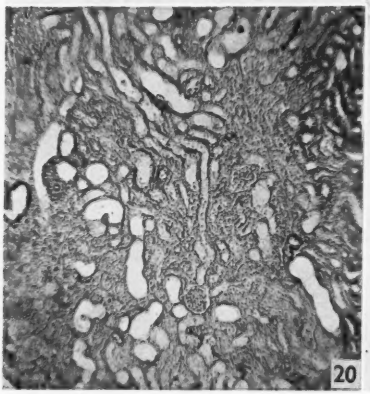
ALBERT AND LEBLOND—AGE CHANGES REVEALED BY CARBONYL REAGENTS

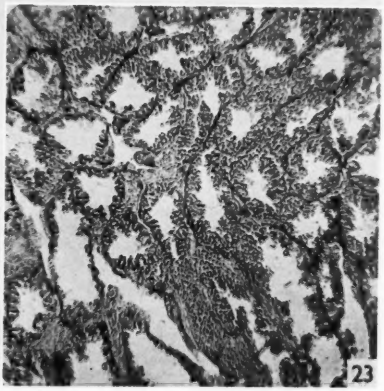












ALBERT AND LEBLOND—AGE CHANGES REVEALED BY CARBONYL REAGENTS

A COMPARISON OF ENDOCRANIAL CAST AND BRAIN OF AN AUSTRALIAN ABORIGINE

By A. D. PACKER, *Department of Anatomy, University of Adelaide*

A comparison of an endocranial cast with the corresponding brain is the obvious method of determining how much accurate information about the brain can be obtained from the cast. Symington (1915) using human European material made such a comparison. His studies showed that very little of the detail of the sulcal pattern was evident on the cast—he was able to identify only the lateral sulcus and its posterior branch, the superior and middle temporal sulci and the sulci related to the orbital surface of the frontal bone. He also showed that the sulcal pattern, especially of the vault, was obscured by the structures lying between brain and skull, viz. meninges, arachnoid granulations, cerebrospinal fluid, cerebral veins and lacunae, venous sinuses and meningeal vessels. On the basis of these investigations he criticized (1916) the interpretations of a number of workers on casts from fossil skulls.

Le Gros Clark, Cooper & Zuckerman (1936) made a similar study in the chimpanzee and concluded that little information about the convolutional pattern of the brain could be derived from the cast—they were able to identify with certainty the rostrum of the lateral sulcus, the fronto-orbital sulcus, the superior and inferior frontal sulci and the inferior pre-central sulcus; vaguely or inconstantly marked were the lunate sulcus, the central sulcus, the parallel sulcus, the angular sulcus and the inferior temporal sulcus.

Both of the above-mentioned investigations showed that there were depressions on the casts not corresponding to the positions of sulci, indicating that the mapping of sulci on an endocranial cast without the brain for comparison would be liable to error.

The endocranial cast of the Australian aborigine has been used in morphological studies, mainly for comparison with other casts, by Keith (1925), Elliot Smith (1928), Kappers (1929), Shellshear & Elliot Smith (1934) and Hirschler (1942); Shellshear (1934) described the cranio-cerebral relations of one cerebral hemisphere. No direct comparison of cast with corresponding brain has been published. Therefore, it seemed of value to make such a comparison, in order partly to bridge the gap between Symington's findings for the European and those of Le Gros Clark *et al.* for the chimpanzee.

MATERIAL AND METHODS

The brain and endocranial cast were from a full-blooded male Australian aborigine aged 64 years. The body had been injected with a solution containing 5 % formalin. Nine days after the injection the skull-cap was sawn through and removed, and the brain removed *in toto*, the brain stem being cut at the level of the foramen magnum. The brain was in good condition with no distortion and no obvious shrinkage; photographs and measurements were made on the day of removal, before any drying could occur.

The dura was stripped as cleanly as possible from the whole of the inside of the skull—some difficulty was encountered in the region of foramina—and a cast was prepared, using melted printer's gelatin. A hole (20 mm. diameter) was bored through the skull near the vertex, the skull-cap was firmly fixed in position and the gelatin poured in through the hole. From the gelatin cast a plaster mould was prepared and finally a plaster cast, using fine dental plaster of Paris in both procedures. The volume of the cast was estimated and measurements taken. Hirschler's base-line joining the hindmost point of the cast to the most medial point of the marking of the fronto-marginal sulcus (B.N.A.) was employed. Using an oblique beam of light the depressions on the cast were carefully marked in pencil; this was done without reference to brain or skull. Tracings of photographs of various views of brain and endocranial cast were then superimposed (with correction for slight differences in size) and the general contours were compared; any sulci corresponding to depressions on the cast were noted, particular attention being paid to the central, lateral and lunate sulci. Obvious eminences on the cast were outlined and compared with corresponding regions of the brain.

The terminology of the brain sulci is in the main that used by Shellshear (1937) in his study of brain morphology of the Australian aborigine.

MEASUREMENTS

The measurements made on brain and cast are given in the following Table. The brain stem was cut in the region of the foramen magnum and the cerebral peduncles were divided at their junction with the pons. The weight includes pia-arachnoid and blood vessels.

	Brain		Cast
Weight	Forebrain and midbrain	920 g.	
	Hindbrain	171 g.	
	Total	1091 g.	
Volume (water displacement)	Forebrain and midbrain	1010 c.c.	1270 c.c.
	Hindbrain	142 c.c.	
	Total	1152 c.c.	
Maximum length	Left side	166 mm.	Left side 173 mm.
	Right side	160 mm.	Right side 166 mm.
Maximum breadth		112 mm.	117 mm.
Base line (Hirschler)	Left side	160 mm.	Left side 166 mm.
	Right side	155 mm.	Right side 162 mm.
Height index (Hirschler*)	Left side	38·1	Left side 45·1
	Right side	37·2	Right side 44·0
Depth index (Hirschler*)	Left side	21·9	Left side 18·1
	Right side	22·8	Right side 18·4

* Hirschler's height index is the greatest height of the brain above the base-line, expressed as a percentage of the base-line. His depth index is the maximum depth of the temporal lobe below the base-line expressed as a percentage of the base-line.

Duckworth (1903) found the average capacity of 150 Australian aboriginal skulls to be 1246 c.c. Davis (1869) estimated the average weight of 17 male brains to be 1197 g. Thus the cast here studied is above average volume but the brain is less than average weight.

GENERAL CONTOUR

A brief description of the contour of the cast is given and mention made of the regions in which the cast does not give an accurate indication of the corresponding surface of the brain.

Superior midline region (Figs. 1–4, 7 and 8; throughout, the odd-numbered figures are of the cast and the even-numbered of the brain)

The frontal lobes of the cast slope upwards from the rounded symmetrical poles with an even convexity (comparable with that of the brain) as far as a centrally placed bregmatic eminence, which is an obvious midline elevation with its centre approximately at the bregma and measuring 45 mm. long by 40 mm. wide—this eminence does not correspond to any elevation of the brain, which is rather flattened in this region. It almost certainly marks the position of the bregmatic 'pool' of cerebrospinal fluid. Arachnoid granulations cause irregularities on its surface.

Immediately behind this 'pool' the contour of the midline is obscured by the hole bored in the skull to make the cast. Just posterior to this region and on either side of the midline are two oval longitudinal elevations, not well defined at their periphery and not symmetrical; their surfaces are made irregular by the markings of several arachnoid granulations. On the left side the approximate size is 50 by 30 mm.; on the right the elevation is slightly longer and rather narrower. The brain in this region shows no definite elevations, although it is at the maximum convexity of curvature at the upper part of the occipital lobes. The elevations are probably due to 'parietal pools' of cerebrospinal fluid.

Behind these eminences the surface of the cast is flattened, but there is no definite depression. The curvature of the corresponding brain surface is regular and rounded, suggesting that the area of flattening on the cast is determined mainly by the presence of the 'parietal pool' eminences lying in front of it.

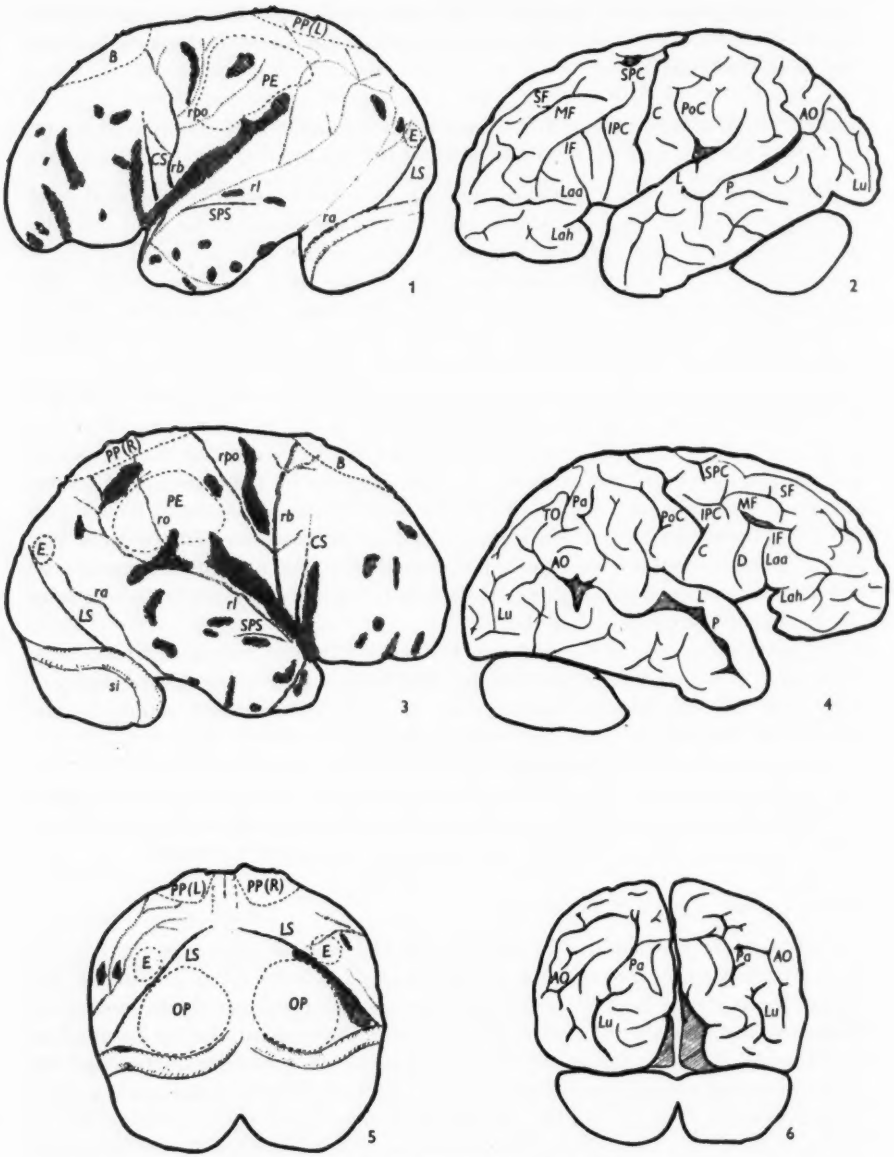
The occipital poles are very prominent on the cast; they are asymmetrical, the left being more pointed and projecting more posteriorly; both poles lie well behind the lambdoid suture markings and overlap the cast of the cerebellum by about 10 mm. There is a comparable asymmetry and overlap in the case of the brain.

Lateral regions (Figs. 1–8, 11 and 12)

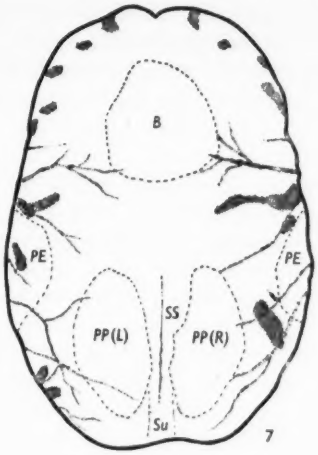
The most obvious features of the cast are the parietal eminences (outlined in Figs. 1 and 3); they are definite elevations on both sides lying just above and behind the posterior limb of the lateral sulcus; at the periphery the eminences are ill-defined, but their shape is on the whole circular. There are similar but less obvious elevations of the brain formed by the lower part of the post-central gyrus and the inferior parietal lobule. The parietal eminence on the right side of the cast is slightly more prominent than on the left, and this difference is present in the brain also.

Also noticeable is the tendency to constriction of the cast immediately in front of the coronal suture markings; the brain is constricted to a similar degree.

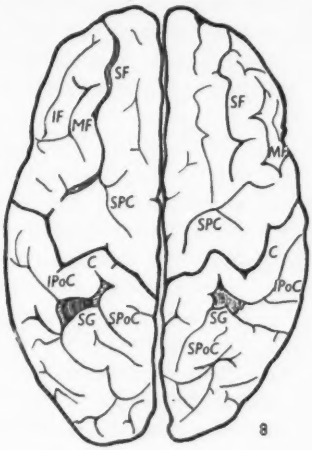
Situated just in front of the markings of the lambdoid sutures and approximately 35 mm. lateral to the lambda are two small rounded elevations of diameter less than



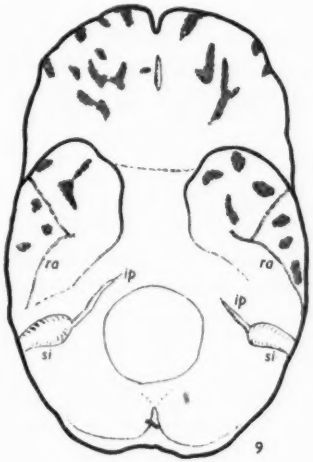
Figs. 1–6. For explanation see end of article.



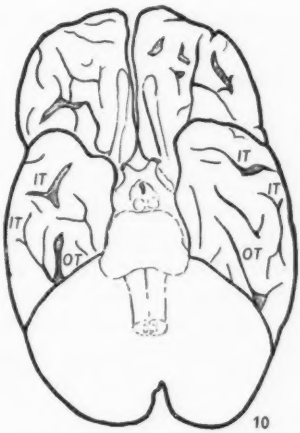
7



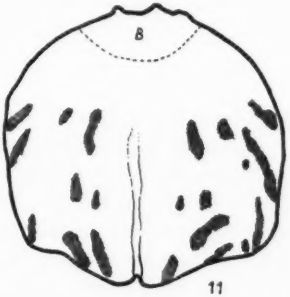
8



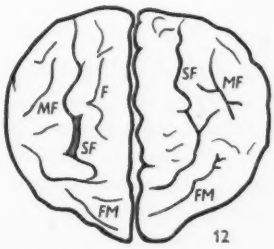
9



10



11



12

Figs. 7–12. For explanation see end of article.

20 mm. They are symmetrical in position, the right is slightly more obvious than the left, but neither is well defined. There are no corresponding elevations on the brain.

The contours of cast and brain over the remainder of the lateral regions correspond fairly accurately and need no further description.

Frontal and inferior regions (Figs. 1–4 and 9–12)

Near the midline the rounded frontal poles of the cast continue inferiorly into a rather blunt orbital keel which projects downwards for only a short distance. Compared with the brain the keel is slightly broader and projects downwards a little more, but it represents quite closely the brain contour.

The orbital surfaces of the cast are concave from side to side and less markedly so from before backwards. As the medial border formed by the keel projects farther downwards than does the lateral, the orbital surfaces look laterally as well as downwards. The lateral border has several notches in it and ends posteriorly in a projection marking the position of the orbital operculum, which is more obvious on the left side of both cast and brain. These opercula are more prominent on the cast and in frontal view give a quadrangular outline compared with the more rounded contour of the brain.

In the brain the insula is partly exposed on both sides, particularly on the right, whereas on the cast the notch at the stem of the lateral sulcus is wider on the left.

The contour of the temporal lobes of the brain is accurately shown by the cast.

SULCAL PATTERN

Frontal region (Figs. 9–12)

On the orbital surface of the frontal lobe of the cast there is some sulcal pattern present, but it does not accurately represent the sulci of the brain—some of the widest sulci not being represented on the cast, while some small but wide depressions on the gyri are well marked on the cast. On the upper and anterior surfaces of the frontal lobe there are several ill-defined depressions on the cast; on both sides, those corresponding to the superior frontal sulci can be made out fairly readily. On the right side comparison with the brain shows that there is a depression on the cast corresponding to a wide longitudinal furrow on the superior frontal gyrus. Two other depressions lie along the line of the front-marginal sulci. None of the other grooves accurately corresponds to a sulcus but several of the smaller ones coincide with short wide depressions on gyri.

Parietal region (Figs. 1–4)

The stem of the lateral sulcus and its posterior limb are accurately indicated on both sides of the cast by a well-marked groove extending to just below the parietal eminence. The anterior horizontal rami are not indicated on either side. On both sides of the cast there is a definite short groove lying just in front of the coronal suture marking: the anterior ascending rami lie mainly just anterior to these grooves although the lower part of the left anterior ascending ramus coincides with the lower part of the groove. It is probably more accurate to say that the groove indicates the depressed part of the brain between the fronto-parietal and frontal opercula. On

neither side of the cast is the central sulcus accurately indicated. On both sides there is a depression lying posterior to the upper part of the bregmatic branch of the middle meningeal artery and in each case the lower part of this groove coincides with the position of the middle of the central sulcus, the coincidence being more exact on the left side. The upper parts of the central sulci lie posterior to the groove. No other grooves of the parietal region accurately correspond to any sulci. There is a definite short depression just above and posterior to the right parietal eminence which corresponds with a partly depressed gyrus (the anterior part of the superior parietal lobule) lying immediately posterior to the upper end of the post-central gyrus. However, on the left side of the brain there is a similar sunken gyrus but no groove on the cast.

Occipital region (Figs. 5, 6)

There is no sulcal pattern obvious in this region of the cast. On the right side there is a groove lying immediately behind and parallel to the lambdoid suture marking, but this does not correspond to any sulcus on the brain. The lunate sulci are situated entirely posterior to the lambdoid sutures. The sulcus on the left side is wide and deep compared with that on the right, but it is not represented on the cast. The lunate sulci are farther from the lambdoid suture than Shellshear (1934) found in his material.

Temporal region (Figs. 1–4, 9 and 10)

In this part of the cast there are some short, relatively well-marked depressions on the lateral and inferior surfaces. They do not lie accurately along the line of the sulci on the right side, but on the left side four such small notches mark the position of the anterior end of the inferior temporal sulcus. The parallel sulci are not represented on the cast, in spite of the fact that on the right side of the brain the sulcus is widely open.

MARKINGS OF VESSELS AND CRANIAL SUTURES

These markings are very similar to those on endocranial casts of European man. They are illustrated in Figs. 1, 3, 5, 7 and 9. The well-marked ridges formed by the middle meningeal vessels are named according to Ruggeri (1913), and the pattern of the ridges corresponds to type I of that author's classification for modern man. The pattern also agrees in basic plan with that depicted by Schepers (Broom & Schepers, 1946).

DISCUSSION

In general, the endocranial cast here described may be said to give a fairly accurate indication of brain shape and contour. The main discrepancies are due to provision for cerebrospinal fluid circulation, the bregmatic and the two parietal 'pools' causing definite elevations near the midline. It is probable that lacunae laterales and cerebral veins as well as arachnoid granulations take part in forming these eminences. The apparent flatness of the cast in the region between the parietal 'pools' and the occipital poles can be interpreted as due to contrast with the adjacent elevations. This interpretation is strengthened by considering the brain contour, which is more

convex than that of the cast in the region concerned. Shellshear & Elliot Smith (1934) have mentioned a lack of development in the pre-occipital region of the Australian aborigine, but it is not clearly evident in this brain. Although there is some fullness of the cast in the region of the coronal suture, there is no definite evidence of any subcoronal stream of cerebrospinal fluid as described and depicted by Keith (1931) for modern man and some lower types.

The sulcal pattern is poorly marked on the cast; this is in general agreement with the other brain/cast comparisons made in the European and the chimpanzee.

A number of short, relatively deep depressions on the cast coincide in position with widely open pits or furrows along the course of some gyri (most obvious in the temporal region), or with widely open parts of sulci (e.g. the left inferior temporal sulcus). These findings support those of Hirschler who has pointed out that widely open rather than deep depressions on the brain are more likely to correspond with depressions on the cast, and that such depressions are of little value in working out the sulcal pattern. On the other hand, a number of depressions on the cast do not appear to coincide with any hollows of the brain surface. Hirschler has also mentioned that a depressed gyrus may cause a groove on the cast, and this is shown on the right side of this cast. However, as the groove is situated between the elevation of the right parietal 'pool' and the right parietal eminence, there must be some doubt about this interpretation; the more so when one considers that on the left side of the cast there is no depression corresponding to a similar sunken gyrus. There are other instances of widely open brain depressions (e.g. the right parallel sulcus and the left lunate sulcus) not obscured by the bregmatic or parietal 'pools' and yet not represented on the cast. Obviously, the various structures and the cerebrospinal fluid lying between brain and skull must play a part as 'obscuring factors'.

The sulcal pattern of the cast is also made more difficult of interpretation by the markings of the meningeal vessels. The main vascular markings are well-defined ridges which give the impression that there is a groove on either side of them. The most obvious example of this is in relation to the marking of the central sulcus whose middle part coincides with a groove between the bregmatic branch of the middle meningeal vessels and the parietal eminence. The lower part of this groove does correspond in position to the middle of the central sulcus, but whether there is a direct relationship seems more doubtful.

Hirschler and others have indicated the difficulty of deciding from a cast the degree of exposure of the insula. This is well borne out here, for, on the cast, the stem of the lateral sulcus is wider on the left side, while, on the brain, the insula (because of the poorly developed orbital and frontal opercula) is more exposed on the right. The explanation of this discrepancy is not apparent.

The brain/endocranial cast studies on primates so far made all show the difficulty of correctly inferring the sulcal pattern from the cast markings, even in those regions where there are well-marked grooves. It seems probable, therefore, that the interpretation of the sulcal pattern on endocranial casts from fossil skulls is likewise liable to error. It is suggested that such casts might well be restudied after an extensive preliminary comparison of brain and endocranial cast in living primates.

SUMMARY

1. An endocranial cast prepared from the skull of a full-blooded Australian aborigine is compared with the corresponding brain.

2. The shape and contour of brain and cast correspond fairly accurately except superiorly, near the midline, where elevations on the cast are produced by collections of cerebrospinal fluid, arachnoid granulations and probably, also, by lacunae laterales and cerebral veins.

3. The sulcal pattern is poorly marked on the cast and few sulci are easily identified; a number of the grooves on the cast do not coincide with the position of sulci. Some of these grooves indicate the situation of short wide depressions on the brain, usually along the course of gyri, but sometimes along sulci.

4. On the other hand, several wide sulci or parts of sulci are not represented on the cast.

5. The markings on the cast caused by vessels and sutures are illustrated. These markings (and the midline elevations) tend to give an erroneous idea of the presence of grooves lying alongside them.

6. The degree of exposure of the insula cannot be inferred from the cast.

7. These results are in general agreement with those of other more recent brain/endocranial cast comparisons in modern man and the chimpanzee.

This work was commenced in 1939 at the suggestion of Prof. F. Goldby, to whom I am indebted for advice and references. It was completed in 1948 with the encouragement of Prof. A. A. Abbie, who gave further advice and further references to literature.

REFERENCES

Broom, R. & Schepers, G. W. H. (1946). *The South African Fossil Ape-Men, The Australopithecinae.* Pretoria: Transvaal Museum.

Clark, W. E. le Gros, Cooper, D. M. & Zuckerman, S. (1936). The endocranial cast of the chimpanzee. *J. R. Anthrop. Inst.* **66**, 249–268.

Davis, G. (1869). Quoted by Kappers (1929).

Duckworth, W. L. H. (1903). Quoted by Kappers (1929).

Hirschler, P. (1942). *Anthropoid and Human Endocranial Casts.* Amsterdam.

Kappers, C. U. A. (1929). *The Evolution of the Nervous System in Invertebrates, Vertebrates and Man.* Haarlem.

Keith, A. (1925). *The Antiquity of Man.* London: Williams and Norgate Ltd.

Keith, A. (1931). *New Discoveries Relating to the Antiquity of Man.* London: Williams and Norgate Ltd.

Ruggeri, V. G. (1913). Über die Endocranischen Furchen der Arteria Meningea Media beim Menschen. *Z. Morph. Anthr.* **15**, 401–412.

Shellshear, J. L. (1934). The cranio-cerebral survey of an Australian aboriginal. *Chin. Med. J.* **48**, 1202–1215.

Shellshear, J. L. (1937). The brain of the aboriginal Australian. A study in cerebral morphology. *Philos. Trans.* B, **227**, 293–409.

Shellshear, J. L. & Smith, G. Elliot (1934). A comparative study of the endocranial cast of *Sinanthropus. Philos. Trans.* B, **223**, 469–487.

Smith, G. Elliot (1928). *Rhodesian Man and Associated Remains.* British Museum (Nat. History).

Symington, J. (1915). The relations of the inner surface of the cranium to the cranial aspect of the brain. *Edinb. med. J.* **14**, 85–100.

Symington, J. (1916). Endocranial casts and brain form: A criticism of some recent speculations. *J. Anat., Lond.*, **50**, 111–130.

EXPLANATION OF FIGS. 1–12

All drawings are reduced to just less than half-size.

Figs. 1, 3, 5, 7, 9, 11 are tracings from photographs of different views of the endocranial cast. Depressions are indicated by cross-hatching; elevations, vascular and sutural markings are also shown.

Figs. 2, 4, 6, 8, 10, 12 are tracings from photographs of comparable views of the brain. Widely open sulci are indicated by shading; the sunken gyri are also shown. The main sulci are labelled according to Shellshear (1937).

Abbreviations

AO	anterior occipital sulcus
B	bregmatic 'pool' eminence
C	central sulcus
CS	coronal suture
D	diagonal sulcus
E	small eminence above lambdoid suture
F	longitudinal furrow on superior frontal gyrus
FM	fronto-marginal sulcus
IF	inferior frontal sulcus
ip	inferior petrosal sinus
IPC	inferior pre-central sulcus
IPoC	inferior post-central sulcus
IT	inferior temporal sulcus
L	lateral sulcus
Laa	anterior ascending branch of lateral sulcus
Lah	anterior horizontal branch of lateral sulcus
LS	lambdoid suture
Lu	lunate sulcus
MF	middle frontal sulcus
OP	occipital pole
OT	occipito-temporal sulcus
P	parallel sulcus
Pa	paroccipital sulcus
PE	parietal eminence
PoC	post-central sulcus
PP (L)	left parietal 'pool' eminence
PP (R)	right parietal 'pool' eminence
ra	ramus astericus
rb	ramus bregmaticus
rl	ramus lambdaticus
rpo	ramus pre-obelicus
ro	ramus obelicus
SF	superior frontal sulcus
SG	sunken gyrus (anterior part of superior parietal lobule)
si	sigmoid sinus
SPC	superior pre-central sulcus
SPoC	superior post-central sulcus
SPS	squamo-parietal suture
SS	sagittal suture
Su	superior sagittal sinus
TO	transverse occipital sulcus

BONES, MUSCLES AND VITAMIN C

II. PARTIAL DEFICIENCIES OF VITAMIN C AND MID-DIAPHYSEAL THICKENINGS OF THE TIBIA AND FIBULA IN GUINEA-PIGS

By P. D. F. MURRAY,* *Department of Zoology, University of Sydney, N.S.W., Australia*

AND

E. KODICEK, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

INTRODUCTION AND EARLIER OBSERVATIONS

In earlier papers (Kodicek & Murray, 1943; Murray & Kodicek, 1946) we briefly reported the formation, in guinea-pigs kept on diets partially deficient in vitamin C, of very extensive diaphyseal thickenings of the tibia and fibula. The present paper contains a more complete report on those changes, which were centred about the middle of the diaphyses. Other structural changes which occurred at the proximal ends of tibial diaphyses in the same material are described in the third paper of this series. The experiments were those described in the first paper of the series (Murray & Kodicek, 1949); they are summarized in Table 1 of that paper.

This is not the first occasion upon which the development of new sub-periosteal bone has been observed in guinea-pigs during prolonged partial deficiencies of vitamin C. Höjer (1924) constantly found osteoporosis of the jaw accompanied by the formation of spongy porous bone on the surface. His illustrations leave no doubt that this spongy bone resembled that described on limb bones in the present paper; indeed, Höjer comments that a similar tissue was formed on the diaphyses of long bones in the presence of a fracture or other circumstances leading to increased periosteal activity.

More recently, Mouriquand and his colleagues (1937, 1938, 1940) have described diaphyseal thickenings as seen in radiographs which leave no doubt of the identity of the phenomenon with that which we have observed, but so far as we are aware they have published no histological study. They, like us, find the thickenings to be formed in prolonged partial vitamin C deficiency but, since they made no fractures, they did not observe the connexion with injury (see below, p. 214). We have not seen the irregular osteophytes which they describe, but as these are said to depend for their formation on the presence of lemon juice in the diet, which we did not use, this is not surprising. Some of their radiographs show the bow-legs and the slope of the proximal epiphysis of the tibia described below.

A number of authors (e.g. Baetjer, 1919; Bromer, 1928, 1943; Scott, 1941; Wimperger, 1935) have described the appearance of very large subperiosteal haematomas on the long bones of children suffering from scurvy (Barlow's disease). These calcify, and probably ossify, after organization. Similar changes were found in monkeys by Hart & Lessing (1913).

* This work was done while the first author was at the Department of Biology, St Bartholomew's Hospital Medical College.

Finally, we are permited to mention a comment by Mr Last, of the Royal College of Surgeons, made after seeing some of our material, that he had seen radiographs which showed diaphyseal thickenings in the legs of scorbutic Ethiopians closely resembling ours taken from guinea-pigs. Of course, similar thickenings are well known in Man under circumstances not suggestive of any vitamin deficiency, as in the chronic periostoses of syphilis, and in obscure circumstances in infants (Caffey, 1946; von Chiari, 1938; Ellis, 1938–9).

EXPERIMENTS, MATERIAL AND METHODS

These were the same as for the first paper of this series (Murray & Kodicek, 1949), and are there described. The most important features of the experiments are summarized in Table 1 of that paper.

MID-DIAPHYSEAL SUBPERIOSTEAL THICKENINGS

Mid-diaphyseal thickenings in normal animals

The subperiosteal thickenings seen in the animals kept on diets partially deficient in vitamin C proved to be an exaggeration of a change seen in the tibiae and fibulae of dietetically normal animals with fractured fibulae. In these animals (group 1 of Exps. 3–6), the radiographs taken at 14 or 19 days after the operation (the earliest, except those made on the day of the operation itself) showed a thickening of the tibial and fibular diaphyses, in the operated legs (Pl. 1, figs. 1, 2B). In the fibula this occurred in the neighbourhood of the callus, and was indeed an extension of it, but might extend for considerable distances above and below the site of the fracture, and was evidently to be identified with the 'distant periosteal reaction' described by Hertz (1936). In the tibia it appeared from the radiographs to be related to the mid-diaphyseal region, where the concavity of the bone is greatest, rather than to the position of the fracture in the fibula. It was nearly always a slight superficial thickening such as is shown in Pl. 1, fig. 1, but in a few cases it was larger.

The radiographs suggested that these thickenings were due to the deposition of new periosteal bone, and this was confirmed by sections (Pl. 1, fig. 5). The mode of their formation in dietetically normal animals was investigated in eight guinea-pigs in each of which a fibula was fractured and which were killed 1, 2, 3, 4, 5, 6, 7 and 9 days later. These animals are included in Table 1 as part of Exp. 1. We studied longitudinal sections which passed through the tibia and fibula of the operated legs, including the region of the fractures and a considerable length of both bones above and below it.

No description will be given here of fracture repair in these animals, because it is hoped that one of us (P. D. F. M.) will discuss certain of its problems later. The conditions foreshadowing the development of the thickenings are seen in the animals killed on the first to third days after the fracture. In these, the periosteum of both tibia and fibula is, over wide areas, lifted away from the bone by oedema and haemorrhage beneath it (Pl. 1, fig. 6). In the specimens killed on the second and third days, cells had filled the space between the fibrous layer of the periosteum and the bone (Pl. 1, fig. 7) and mitoses were numerous among them. In the animal killed at 4 days there was very early bone formation in the enlarged cambial tissue thus

formed on the lateral surface of the tibia. The first new bone contained in its matrix great numbers of small basophil granules. These were also formed in the callus which later developed about the point of fracture, and in the subperiosteal thickenings of animals on diets partially deficient in vitamin C; they will be mentioned again in the latter connexion. At 4 days, the formation of the new subperiosteal bone had begun on the fibula (but not in the immediate neighbourhood of the fracture, where the periosteum was dead or absent, having presumably contracted away), and on the adjacent lateral surface of the tibia (Pl. 2, fig. 8). In succeeding days, there was further development of this bone, resulting in the formation of short thick trabeculae extending from the old surface of the diaphysis to the fibrous layer of the periosteum (Pl. 1, fig. 5). It is noteworthy that the new bone is not a simple addition to the old, formed by an increased rate of deposition of compact bone resembling that already forming the diaphyseal wall, but is architecturally a new structure.

In animals killed many days (e.g. 94, 102) after the operation, and kept throughout on a diet fully supplemented with the necessary vitamins, the trabecular bone thus formed was found to have been made compact by deposition on the trabeculae, the spaces between them being thus reduced to no more than vascular channels. Nevertheless, the irregular orientation of the fibrillar structure still made it easy to distinguish between the new bone and that of the original diaphysis, with its much more regular structure. Frequently, layers of regular bone, in all respects normal, had been formed externally, by deposition on the surface in a cambial layer which was no longer abnormally thickened. The bone earlier formed in the widened cambium, after injury, could then be seen sandwiched between two layers of more regular bone, one situated internally, and the other externally to it, or it might be found lining the marrow cavity if the original diaphyseal wall had been completely removed from within in the course of normal growth of the tibia.

Animals on diets deficient in vitamin D

These animals showed no differences of any kind from the normal animals.

Mid-diaphyseal thickenings in animals kept on diets partially deficient in vitamin C

In these animals the diaphyseal thickenings might be absent, and when present might range from thickenings as small as in the animals on normal diets to very large indeed (Pl. 1, fig. 2D; Pl. 2, fig. 9). Table 1 summarizes the occurrence, in the partially deficient groups of Exps. 3–6, of thickenings which were larger than were usually seen in animals of groups 1 and 2 of these experiments (which had ample vitamin C throughout). The Table includes both mid-diaphyseal thickenings of the tibia and fibula, and similar thickenings at the proximal end of the diaphysis.

Table 1

(Explanation in text)

		5 cases out of 6 legs
Exps. 3 and 4, group 3		
4	9	28
Exps. 5 and 6, group 3	10	19
4	9	20
5	1	8
6	2	8

The structure of the thickenings

The new bone which formed the thickenings was deposited wholly on the surface of the old. The thickenings were thus periosteal structures, and were therefore limited to the diaphyses of the bones.

In the normal bone of the diaphysis of the guinea-pig's tibia or fibula, the fibrous architecture is longitudinally arranged and shows a lamellar structure only in part. The remainder of the bone is, in the words of Weidenreich (1923, 1930), and other German authors, a parallel-fibred fine fibrous bone. The new bone is not a mere continuation and enlargement of the existing structure, but has a new and quite different architecture (Pl. 2, fig. 11) which is abruptly joined to the old and does not pass into it by gradual transition. Usually, one or several lines of 'cement' intervene at the periphery of the old bone and demarcate it from the new. Often, however, recognition of the boundary between the old and the new is made difficult by the extreme degree of porosis which the old may suffer, for it may be so dissected that its fragments simulate trabeculae of the new bone, and this resemblance may be increased by the deposition of narrow layers of new bone over the surfaces of the remaining parts of the old.

In transverse sections of the diaphysis of an affected bone (Pl. 2, figs. 9–11), the thickening usually consisted of rather narrow bony trabeculae radiating to the periosteum and joined by occasional cross connexions. Tangential sections (Pl. 2, fig. 12), however, showed that the radiating trabeculae were really flattened plates running longitudinally, at right angles to the surface of the old bone, from it to the periosteum. How far this verbal description exaggerates the regularity of the structure will be seen from the photographs.

Most of our sections showed the new radiating trabeculae arising from the compact but now porotic original bone of the diaphyseal wall, but in some cases the resorption of the compacta from the side of the marrow, occurring in the normal course of growth, combined with the porosis and with the superficial resorption which often precedes the development of the new trabecular bone (see below), led over wide areas to the total destruction of the old bone. The diaphyseal wall then consisted in these regions entirely of the radiating trabeculae. In other cases it could be seen that the destruction was not quite complete, an almost entirely trabecular wall being completed by a narrow zone of old compact bone (Pl. 3, fig. 13).

At the surface of the new bone there was often found some attempt to form a peripheral bony shell joining the ends of the radial trabeculae; this structure was usually very incomplete (Pl. 3, fig. 14).

The surface of the thickening was covered by the fibrous layer of the periosteum. Beneath this, there might be groups of cells clustered round the ends of trabeculae still growing in length, or covering the surfaces of the developing peripheral bony shell. Where the general connective tissue of the limb, outside the periosteum, was in the hyperplastic condition described in the earlier paper of this series, the fibrous layer of the periosteum tended also to be involved, and might in part disappear, dissolving into the cellular and fibrillar tissue which replaced the normal soft tissues of the leg. Such dissolution of the periosteum, when seen at all, was confined to part only of the circumference in transverse sections. Where it occurred, there was no sign of any attempt by the new bone to form a superficial 'shell'. The trabeculae

appeared to radiate outwards into the general connective tissue of the leg, but in fact they did not become longer in these regions than elsewhere, i.e. they did not pass beyond the zone which could be recognized, by extrapolation from the remaining parts of the periosteum, as 'belonging' to the bone.

The tissue between the trabeculae of the new bone was a loose connective tissue with a delicate fibrillar stroma whose fibrils ran into the bone. The cells had usually the appearance of fibroblasts; many of them were flattened against the trabeculae. In places cells were found against the bone in more or less regular rows, or grouped around the ends of the trabeculae. There can be no reasonable doubt that these were osteoblasts, but they often lacked the regular form and arrangement of normal osteoblasts, a feature which has often been reported in animals suffering from various degrees of scurvy.

Vascularity of the thickenings

A remarkable feature in many of the fully developed thickenings in animals which had been for long periods (up to 17 weeks) on the experimental diets was the small number of blood vessels in the connective tissue between the trabeculae. In some, there were a few channels resembling capillaries but without red corpuscles, while others seemed to be completely, or almost completely, avascular. This condition proved to be associated with the connective tissue hyperplasia in which, as described in the earlier paper, part or the whole of the musculature of the limb was locally replaced by a loose connective tissue in which blood vessels were scarce, and in which many of those which were present were structurally abnormal and contained few or no corpuscles. There were seven limbs having mid-diaphyseal thickenings which were partly or wholly vascularized, indeed in some hyperaemic, and in each case the connective tissue of the limb was either not hyperplastic or only slightly so. It thus seemed likely that the avascular condition of the new bone was a result of the similar condition in the soft parts of the limb, from which the normal periosteum probably receives at least many of its blood vessels. It may be noted that the vascularity of the old diaphyseal wall and of the marrow was not affected in this condition.

The seven limbs just mentioned, which had well vascularized subperiosteal thickenings, were from animals that lived on the experimental diets for the following periods: 22, 23, 37, 39, 55 (two animals) and 77 days. This seemed to suggest that thickenings when first formed might be well vascularized and lose their vessels later, perhaps with the development of the connective tissue hyperplasia. We therefore studied the vacularization of the developing thickenings in the tibiae of dietetically normal animals having fractured fibulae (material from Exp. 1, see Table 1, Part 1), and of the partially deficient animals in Exp. 9 (see Table 1, Part 1). In the dietetically normal animals killed at 4 and 5 days after the operation, the tissue of the widened cambial layer of the periosteum was almost avascular, the vessels of the periosteum having failed to keep pace with its growth. At 6 and 7 days, the tissue, in which young bone was developing, showed sparse capillary vessels containing blood corpuscles, and by 8 days the new tissue was well vascularized. Thus the early failure of the periosteal vascular system to keep pace with the changes in the cambial zone was quickly rectified. In later stages, the thickenings of the dietetically normal animals were always well vascularized by blood vessels between the trabeculae

or, in the oldest specimens, in vascular canals in the now compact bone. In the partially deficient animals of Exp. 9, on the other hand, the vessels of the periosteum failed altogether to grow *pari passu* with the thickening of the cambial layer and the growth of the new bone, which therefore developed from the beginning in an almost completely avascular bed. It should be added that, in all cases in Exp. 9 in which new bone formed, the soft tissues of the limb either showed hyperplasia of the connective tissue, or degenerative changes in the muscles which, as described in the earlier paper of this series, are known to precede the hyperplasia.

The formation of new bone in an avascular bed has theoretical interest, for the observation that, in normal callus formation, cartilage appears in avascular regions while bone forms in regions well supplied with vessels, has led authors (e.g. Wurmback, 1928) to regard vascularization and the lack of it as the factor determining the differentiation of the two sorts of tissue. The present observation shows that a rich vascular supply is not a necessary condition of bone formation.

Fine structure of the new bone

The bone which makes up the trabeculae of the thickenings could usually be seen to be of two kinds which we shall distinguish as 'fibrillar' and 'granular' bone (Pl. 3, figs. 15–17). Granular bone was found forming a core to the trabeculae, or as isolated islands in them. The surfaces of the trabeculae were covered with fibrillar bone which must, therefore, have been deposited later than the granular. The granular bone had the following character. The lacunae were of large and irregular form, being produced into processes from which it was rarely possible to see that tortuous canalicules ran off into the matrix. Examined with a high power, the matrix revealed itself to contain, or indeed largely to consist of, great numbers of small rounded granules (Pl. 3, figs. 15, 16). These were basophil; they, or possibly a substance forming a film over their surfaces, stained with haematoxylin, and they thus gave the tissue a tendency to basophilia which helped to accentuate its contrast against the fibrillar bone which covered the granular bone and stained with eosin. The use of Wilder's method for the silver impregnation of collagen fibres showed that the granular bone contained few fibres, and in places apparently none. Examined with the aid of polarized light, the granular bone was not birefringent (after decalcification). The granules were best demonstrated with Giemsa, which gave them a red-purple colour and showed that they lay in a matrix which stained with eosin. Nothing is known of the nature of the granules; they have been observed in various situations by other authors, as by Weidenreich (1930), who considers that each granule represents a site from which the bone mineral, deposited in molar rather than in molecular dispersion, has been dissolved away in decalcification, leaving behind the organic matrix whose staining properties its earlier presence had in some way modified.

The formation of granular bone was not a result of the partial deficiency of vitamin C, for in dietetically normal animals the first bone to appear in callus formation, and in the development of the thickenings, was of this character, and it was then overlaid by fibrillar bone.

The fibrillar bone, in both normal and vitamin C-deficient animals, had the structure and birefringency of normal bone.

Development of mid-diaphyseal thickenings in partially vitamin C-deficient animals

Experience showed that the development of large subperiosteal thickenings was most likely to occur in the tibiae and fibulae of legs whose fibulae had been fractured while the animal was on a diet partially deficient in vitamin C, and radiographs and other evidence indicated that early stages would be found in the first 3 weeks after infliction of the injury. Accordingly, Exp. 9 was performed. A fibula of each animal was fractured and the animals killed at intervals. The material obtained was studied mostly in transverse sections because we wished to know whether a swelling of the periosteum preceded the appearance of the new bone, and in longitudinal sections it is easy to be deceived into a false impression of the width of the periosteal tissue. We took sections from positions estimated to be close enough to the fracture to be within the region in which we might expect the periosteum to be affected, but not so close to it as to be within the region in which the periosteum was killed. In addition, some series were sectioned longitudinally.

The sections showed that the formation of a thickening can be arbitrarily divided into five stages. Although the histological picture showed that these stages must follow one another, nevertheless, the last stage could be found so soon after the injury that it could not possibly have developed as a result of its infliction; for example, in one of the legs fixed at 3 days after the fracture. In such cases the bony thickening must have begun to develop before the fracture occurred; this is not surprising, for other experiments showed that as great thickenings could develop in partially deficient animals which had not suffered any intended injury as in those which had (see below). A thickening may continue to grow peripherally for many weeks after the operation and in such cases all the stages may be found, from stage 3 just below the periosteum to stage 5 close to the old bone.

The five stages will be described in order.

Stage 1. This stage was seen in material from legs fixed at 3 and 5 days after the fracture. The condition of the periosteum resembled that described in normal animals in the first days after fracture. It appeared detached from the bone and it was often haemorrhagic. This stage was one of passive injury; it began to give way to the events of the second stage in some of the 3-day material. Both tibia and fibula often showed signs of resorption at their surfaces, with osteoclasts in Howship's lacunae.

Stage 2. This stage was characterized by the formation of a loose and oedematous connective tissue beneath the fibrous layer of the periosteum and filling the space between it and the surface of the bone (Pl. 3, fig. 18). The tissue consisted of rather star-like cells in a delicate fibrillar stroma; it might show a radial orientation of its fibrils and cells, recalling that described above for the bony trabeculae soon to be formed, or it might show little or no regularity. Many of the cells were in mitosis. Cell divisions occurred much less frequently, if at all, in the fibrous layer of the periosteum, but the histological picture suggested that the fibrous layer might loosen on its inner face and some of its cells be added to the tissue below. Because of its position between the bone surface and the fibrous layer of the periosteum, this tissue was evidently an enlarged periosteal cambium.

Evidently, the enlarged cambium had formed in the space created in stage 1 by

the separation of the periosteum from the bone. Such a space could only exist if either the periosteum had stretched to cover a longer circumference or if the circumference of the bone had lessened, or if both occurred. It is certain that there was in many cases a decrease in the circumference of the bone, for the histological picture sometimes showed very clear evidence that there had been resorption of bone from the surface; the surface was jagged and osteoclasts were seen lying in Howship's lacunae (Pl. 3, fig. 19). The occurrence of such superficial resorption was, however, not invariable, and even if it might sometimes be sufficient to account for a rather narrow although widened zone, formed within a few days of the injury, between the fibrous layer and the old bone, it certainly could not account for the great width of the space which the new bone eventually occupied. In order to do so, the resorptive process would have had to remove the whole thickness of the original diaphyseal wall, which did not happen. There must, therefore, have been both a reduction in the width of the bone, caused by superficial resorption, and an increase in the circumference of the periosteum; and it is the latter which accounts for by far the larger part of the space eventually occupied by new bone.

Stage 3. In the diffuse mesenchyme-like connective tissue into which the cambial zone of the periosteum transformed itself in stage 2 there now appeared groups of larger cells with dense cytoplasm and rather big vesicular nuclei (Pl. 4, fig. 20). These were found near the fibrous layer, near the bone, or anywhere between the two. They were osteoblasts concerned in the formation of the new bone; but for their position and known later history they would not have been recognized as osteoblasts, being still more like fibroblasts than like the osteoblasts of later stages in bone development. They seemed to be formed by a differentiation from other cells resembling mesenchyme cells or fibroblasts, rather than by the division of pre-existing differentiated osteoblasts, for there were all transitions between them and the other cells of the widened cambial zone, and between them and the flattened cells of the fibrous layer.

Stage 4. In this stage there began the formation of a tissue which was evidently identical with the granular bone which we have described as forming the cores of trabeculae in later stages, and which was also the first bone formed in the callus of fracture repair in normal animals. It appeared among and between the cells described above as forming groups in the widened cambial zone, and seemed to consist of a homogeneous or fibrillar matrix in which the basophil granules were deposited. It was usual for the group of cells among which the granular bone formed to be arranged radially (Pl. 4, fig. 21), thus forecasting the radiating architecture seen in the new bone in later stages.

Stage 5. During osteogenesis in the enlarged cambial zone, the formation of granules was a feature of early stages, and at any one point on a developing trabecula soon gave place to the deposition of fibrillar non-granular bone such as is found in the normal skeleton. This was the beginning of stage 5. Very often granular and non-granular bone were separated by a cement layer, but this was not always so, and sometimes a few granules could be found in the young non-granular bone; one might thus be led to suppose granular bone to be a stage in the formation of fibrillar bone, or vice versa. This was refuted by the persistence, in animals killed 3 months after fracture, of granular bone in the cores of the trabeculae, while the more recently

formed bone covering their surfaces was non-granular. The opposite suggestion, that non-granular bone developed into granular, or that the granules formed in it when it became necrotic, is put out of court by the early formation of granular bone, with the granules present from the first, before the fibrillar bone appeared.

The cells concerned in the formation of non-granular bone were often regular series of typical osteoblasts, and were always more osteoblast-like than those found on the surface of developing granular bone.

Porosis and the new bone

The well-known porosis of scurvy (Pl. 2, fig. 9) had appeared in all the sectioned bones of partially deficient animals of Exps. 3–6, except in two or three from animals which died before it developed. It was usually considerable, the old diaphyseal wall showing many large and irregular cavities. Sometimes the porosis was so great as almost totally to destroy the old diaphyseal wall. Osteoclasts were often present, attached to the walls of the cavities, and suggesting that resorption was still going on, but in other cases the cavities showed that there had been deposition of bone on their walls and rows of osteoblast-like cells supported this. One would be inclined to suppose that the resorption must have occurred during the days of total deprivation of the vitamin, and that the deposition had been made possible later, when limited supplies of ascorbic acid were allowed, but this is refuted by the absence of porosis in those which died soonest, and by Exp. 9 (see Table 1, Part 1), in which porosis did not begin till long after the end of the total deprivation.

One of the most remarkable features of the histological picture was the limitation of porosis to the old bone, the new bone being free of it and showing few or no osteoclasts. This does not mean that resorptive processes were completely absent from the new bone, for occasionally one could find indications that slight resorption had occurred, but that the large cavities made in the old bone were not seen in the new, and that what resorption did occur was balanced by deposition. Exp. 9, in which Exps. 5 and 6 were repeated with only unimportant differences, except that the animals were killed sooner and at regular intervals, and that groups 5 and 6 were omitted, threw light on the time relations between the formation of the thickenings and the development of the porosis. In this experiment, in which the last animal was killed at 44 days, porosis never amounted, in the tibia, to more than the appearance of a few large holes seen in cross-sections of the wall; we did not study the fibula in this respect, thinking that the presence of a fracture in that bone would probably influence the onset of porosis in it. Our sections revealed no evidence that porosis had begun in any of the three animals killed at or before 19 days but suggested that it was beginning in two of those killed at 31 days, and in nine out of the ten killed at 34 days and later. Subperiosteal thickenings were found in all animals killed at 37 days and later, and in some of those killed earlier. Thus, the development of the thickenings occurred simultaneously with early stages in the porosis of the old bone. It is, however, true that the development of the new bone was well advanced by the end of the experiment at 44 days, and at this time the porosis was still only beginning, and most of the new bone seems to have been formed before the active porosis whose results were seen in the animals kept for much longer periods (Exps. 3–6). In most of this older material, the histological picture

suggested that, at the time of fixation, the trabeculae were no longer growing in length though there might be evidence of slow deposition of fibrillar bone on the surfaces of the trabeculae. Nevertheless, in some examples, the sections showed quite clearly that the subperiosteal trabeculae were still growing at death, although the old diaphyseal wall was riddled with large cavities and some of these were being enlarged by osteoclasts.

Thus the new bone is not only immune, at least relatively, from the porosis which destroys the old, but can continue to be formed even when the porosis of the old is advanced. This may be connected with the poor vascular supply of the new bone, but we could not find satisfactory evidence on this point.

Diaphyseal thickening and injury to the bone

The difference between the thickenings formed on the tibia and fibula of dietetically normal animals with fractured fibulae, and the much greater development of new bone, worthy to be called 'hyperostosis', which appeared in many animals on diets which were partially deficient in vitamin C, was quantitative and not qualitative. The difference between the two dietary groups was in the thickness of the new bone which in the animals on partially deficient diets usually greatly exceeded that in the dietetically normal animals. In addition, the trabeculae of the new bone were usually thinner, longer, more regularly orientated, and more sharply differentiated into granular and fibrillar bone in animals on partially deficient diets than in those on normal diets.

Thus the partial deficiency of vitamin C tended to cause an exaggeration of a structural change which, in dietetically normal animals, did not occur at all except after operative interference. The combined action of the two factors is emphasized by comparing Exps. 3 and 4, in which the deficiency did not begin till 15 days after the infliction of the injury, with Exps. 5 and 6, in which the deficiency began more than 3 weeks before the injury. When the time of origin and the growth of the thickenings were studied in the radiographs it was found that in Exps. 3 and 4 the thickenings began soon after the infliction of the injury, and before the diet was made partially deficient (as in animals on fully supplemented diets), but only went on to become extreme after the supply of vitamin C was reduced. That is to say, the relatively slight thickenings which formed after the operation, on the fully supplemented diet, went on to become hyperostoses under the influence of a partial deficiency of vitamin C. In Exps. 5 and 6, on the other hand, the partial deficiency of the vitamin did not induce the formation of thickenings until after the operation, but the thickenings which then formed tended to become extreme more rapidly than did those in Exps. 3 and 4.

It was thus the operation which determined the occurrence of the thickening at all, but the partial deficiency of vitamin C which caused its enlargement into a 'hyperostosis'.

In both dietetically normal and partially deficient animals it appeared to be unimportant in the production of the thickenings whether the attempt to fracture the fibula was successful or not. Combining all dietetic groups of Exps. 3–6, the fibula was successfully fractured in thirty-two limbs and, among these, subperiosteal thickenings formed in eighteen, while the attempt to fracture the fibula failed in

thirty limbs and subperiosteal thickenings formed in fourteen. The difference is obviously insignificant.

Although in animals on normal diets there was never any development of subperiosteal thickenings on the bones of unoperated legs, this did occur among the partially deficient animals. In Exps. 3 and 4, out of twenty-five operated legs of partially deficient animals, ten (40 %) showed marked mid-diaphyseal thickenings of the tibia, fibula, or both, and out of eleven unoperated legs such thickenings occurred in two (18 %). In Exps. 5 and 6, out of nineteen operated legs, thirteen (68 %) showed mid-diaphyseal thickenings, and six (17 %) unoperated legs out of thirty-five showed similar thickenings. In Mouriquand's experiments there was no operative interference.

Formation of the widened cambium

We have seen that the formation of the widened periosteal cambium is a result of the detachment of the periosteum from the bone. There was an obvious resemblance between mid-diaphyseal thickenings and the callus which forms around a fracture in an otherwise normal animal; indeed, when such a callus was present the thickening was an extension of it. A number of students of fracture repair have emphasized the importance of periosteal detachment for callus formation. Thus, Lexer (1922), Koch (1924) and Haldeman (1932) regard its separation from the cortical bone as a necessary condition of callus formation, and the first two, if not the third, consider the amount of callus formed to be proportional to the degree of the separation, always provided that the periosteum retains its connexion with the surrounding soft tissues from which it receives most of its blood supply. It is well known that periosteal detachment occurs in acute scurvy. Meyer (1928), in his study of acute scurvy in guinea-pigs, commented that 'the periosteum usually can be stripped with greatest ease', mentioning particularly the mandible. We examined bones of three animals in acute scurvy, decalcifying three tibiae and examining cross-sections cut with a razor; the periosteum appeared to be completely detached over wide areas. No injury had been inflicted. Meyer attributed the separation of the mandible from the periosteum to resorption of bone from the surface, and, as stated elsewhere, we found that such resorption occurred from the surfaces of limb bones. It is perhaps significant that in Exp. 9 (see Table 1, Part 1) the transverse sections of tibiae of animals killed in the first 5 days after fracture of the fibula showed the periosteum detached from the tibia; this had occurred before embedding and sectioning, for it was seen in the decalcified material before these processes were begun, and was therefore not an artefact caused by them. Later, after the development of the enlarged cambial tissue, no detachment was seen. One cannot help suspecting that it may have been caused by resorption of superficial layers of the bone, after operation; re-attachment occurred when the subperiosteal space was filled by the enlarged cambium.

Because of the known inability of scorbutic animals to make collagen fibres, by which the fibrous layer of the periosteum is attached to the bone, it is easy to understand how, even in a moderate scurvy, this layer might have become detached from the bone by any factor tending to have such an effect, and would then have been less readily attached to it again. Among such factors may perhaps be included the fracture forceps, whose use may perhaps drag on the periosteum of both bones,

tending to detach it. However, the fracture forceps can have been only a contributory factor, for the thickenings sometimes developed when there had been no operation. This only happened in partially deficient animals, in both our experiments and those of Mouriquand. We think it possible that a mechanical factor operating in these (and other) cases may have been the pull of muscles on the periosteum, especially when the animals resisted anaesthesia, before the taking of the radiographs, by vigorous kicking. This is perhaps supported by the frequent observation of thickenings which had grown in steps, and showed an older zone of trabeculae which had been closed peripherally by a shell, and external to this a new series of younger trabeculae radiating towards the fibrous layer. Each such 'step' could have been caused at the taking of one of the photographs. Follis (1943) has shown that the immobilization of a limb prevents the microfractures which, in scorbutic animals, otherwise occur close to the epiphyses.

In addition to the direct action of the forceps and contracting muscles, and perhaps more immediately important than either, was the oedema which followed injury to the fibula and which provided a fluid which, along with some haemorrhage, occupied the widened subperiosteal space.

We know that the mid-diaphyseal thickenings occurring in the tibiae of dietetically normal animals after operation were produced by the same detachment, oedema, cambial proliferation and bone formation, as in the partially deficient animals; and we must suppose that its lesser degree reflected a periosteum whose collagen fibres were less readily broken and more readily reformed, a bone whose surface layers were less readily resorbed, and a vascular and metabolic condition in which the oedema caused by injury was less, and was more rapidly drained away, than in animals provided with ample vitamin C.

The determination of the architecture of the new bone

The formation of an abnormal bony architecture of a regular character makes it possible to investigate the conditions of its formation in the hope of throwing light on the mechanism which brings about the development of such structures. Such a regular architecture is provided by the radiating trabeculae of the mid-diaphyseal thickenings.

We have here two problems: (*a*) that of the factors determining whether periosteal growth of a bone in thickness shall be by the apposition of new compact bone, as normally happens, or by the formation of trabecular bone, as in our thickenings, and (*b*) that of the factors determining the orientation of the trabeculae.

In normal growth in width of the diaphysis, new compact bone is deposited by a periosteal cambium which consists of little, if anything, more than a single layer of osteoblasts, with the fibrous layer immediately outside it. Similarly, when in one of the subperiosteal thickenings the growth of the trabeculae has brought their ends against the fibrous layer of the periosteum, the circumferential bony shell is formed (Pl. 3, fig. 14), and, evidently, continued growth of the shell would produce a zone of compact bone. Indeed, after return to a diet containing ample supplies of ascorbic acid, circumferential layers of compact bone are formed (see below). In the formation of both the 'shell' and of these layers of compact bone, the condition of the periosteum resembles the normal: the bone is formed by a narrow cambial layer

beneath an immediately apposed fibrous layer. Thus, the formation of trabecular or of compact bone depends on the dimensions of the tissue in which the bone is developing. If osteogenetic cells are scattered, as it were in three dimensions, through a widened cambial tissue, trabecular bone is formed, but if the fibrous layer of the periosteum holds them in a single sheet against existing bone, they must evidently give rise to compact bone.

A second factor affecting the compact or trabecular nature of the bone in these experiments is the nutritional state. Thus, the trabecular new bone of the thickenings was made compact when the supplies of ascorbic acid were increased to 10 mg. daily, and the compact bone of the diaphysis was made trabecular by porosis during the prolonged partial deficiency.

The walls of long bones are compact because the periosteal deposition of bone here occurs in a narrow cambium held against the old bone, while endosteal bone, unless its formation is limited to apposition against the inner surface of the diaphyseal wall, is usually trabecular.

Turning to the problem of the orientation of the radiating trabeculae of the thickenings, one of us (Murray & Selby, 1930; Murray, 1936) has earlier discussed the formation of similar radiating architectures in other bones (the limb bones of normal chick embryos, of chick embryo limb bones becoming bent while developing in chorio-allantoic grafts, and the bent limbs of chondrodystrophic chickens, Landauer, 1927). The opinion was expressed that the radial orientation of the trabeculae was in those instances to be referred, as Studitski (1934) suggested in some of his experiments with chorio-allantoic grafts, to tensions set up, by the growth in width of the epiphyses and the curvature of the shafts, between the fibrous layer of the periosteum and the cartilaginous or bony shaft. When that discussion was written, however, there was very little evidence that such tensions existed at all. Sections made from the material of Exp. 9 (see Table 1, Part 1), in which we studied the development of the new bone in animals on partially vitamin-C deficient diets, showed young trabeculae of bone radiating from the old diaphyseal wall towards the fibrous layer of the periosteum, but ending at various points in the widened cambium, and then being continued to the fibrous layer by strands of collagen fibres (Pl. 4, fig. 22, arrows) about which were grouped cells which were shown, by comparison with those on the bony parts of the trabeculae, to be osteoblasts. The histological picture left no room for doubt that the bony trabeculae were growing outwards along the collagen fibres and owed their radial arrangement to that of the collagen fibres which provided them with a scaffolding. It has been shown by a number of workers (e.g. Weiss, 1928, 1929, 1933; Levy, 1904) that collagen fibres develop along lines of tension. In our material, the oedematous condition of the subperiosteal tissue separated the periosteum from the bone and set up between the two just such tensions as Studitski imagines, and along these tensions the collagen fibres developed. Finally, the sections show that it is along the radiating collagen fibres that the bony trabeculae form.

The effect of restoring adequate supplies of vitamin C

In Exp. 7 (see Table 1 of Part 1) the guinea-pigs, after deprivation of ascorbic acid for 10 days, were allowed 0·5 mg. ascorbic acid daily for 55 days, and thereafter were given 10 mg. of ascorbic acid, and cabbage, daily until they were killed. We then

investigated the thickenings which had been first formed in the period of partial deficiency. Since we had not at the time appreciated the importance of injury in causing subperiosteal thickening, no fractures were inflicted; this was unfortunate because it reduced the number of markedly thickened bones available for study after 'cure' of the partial deficiency. We obtained sections of four tibio-fibulae from three animals. These all showed thickenings with the usual radiating architecture, but they differed from those seen in animals which died or were killed during the partial deficiency in the much greater thickness of the trabeculae, and corresponding reduction of the intertrabecular tissue. The originally trabecular thickenings had in fact been transformed into compact bone, while retaining clear indication of their original architectural character (Pl. 4, fig. 23). This change was brought about by the deposition of additional fibrillar bone on the surfaces of the trabeculae. Thus, the chief effect of the additional vitamin C on the new bone was to increase its density.

There was, however, another effect which was not seen in animals which were killed, or died, while still on the partially deficient diet. This was the appearance, in the consolidated mass of new bone, of a small number of holes (Pl. 4, fig. 24) obviously made by resorption and often containing osteoclasts. These holes were present in all four specimens, and in size and number recalled the early stages in scorbutic porosis seen in animals of Exp. 9 (see Table 1, Part 1). The amount of bone which had been removed must have been negligible, but of course it might have become a more important quantity if the animals had been allowed to live longer.

When the superficial region of the bone, close beneath the periosteum, was examined, we found clear evidence that its thickness had been reduced by resorption from the surface (Pl. 4, fig. 25). This was very obvious in one specimen particularly, in which the radiating trabeculae were sharply cut off just beneath the periosteum, where osteoclasts were present, and the picture was one typical of a resorption surface.

In parts of this specimen, and in large areas of the surfaces of others, the abrupt and jagged peripheral ends of the partly resorbed trabeculae did not reach the surface, but were buried beneath a superficial layer of bone developed as a circumferential subperiosteal sheet (Pl. 4, fig. 23), such as is deposited on the surfaces of bones in normal growth. This superfical bone must have been formed after the development of the trabecular thickenings, because it overlay a surface from which part of the trabecular bone had obviously been resorbed (like an 'unconformity' in Geology). Since nothing of the sort was ever seen in animals killed while on the partially deficient diet, it must have been formed after the enrichment of the diet with the full supplement of the vitamin.

Turning to the bone lining the marrow cavity, we expected to find the original diaphyseal cylinder but with the porosis cavities more or less completely filled with new bone. Instead, we found compact bone showing no convincing sign that it had ever suffered porosis at all, and this bone covered sharply broken off and jagged inner ends of the trabeculae (Pl. 4, fig. 23). It is evident that, at some time after the formation of the trabeculae, resorption from the marrow cavity destroyed both the old bone of the diaphyseal wall (which must have been porotic) and the inner ends of the trabeculae. This may have happened during the partial deficiency, for we have

other specimens fixed during this period in which endosteal resorption had removed almost the whole of the original cylinder, leaving a wall composed entirely of radiating trabeculae (Pl. 3, fig. 13). Of course, resorption from the marrow is a necessary concomitant of normal growth. After this resorption, the new internal compact bone was then formed.

Thus, the restoration of normal dietary conditions led to a strengthening of the bone composing the shaft by the conversion of trabecular into compact bone, and to a reduction of the abnormal thickening of the wall by resorption from both its outer and inner surfaces. That such a reduction in thickness of the wall did actually occur was confirmed by the radiographs. It was not reversed by some increase in thickness caused by the formation of compact bone both internally and externally, and which may reflect a complex remodelling of the form of the bone as a whole. It must be remembered that, so long as an element is growing in length, the bone at any level in the shaft is in process of becoming the bone at another level (i.e. further from the growth cartilage which formed it), and that the width of the shaft, the thickness of the bony wall, and the width of the marrow cavity, are different at different levels. In particular, the wall becomes thicker and the marrow cavity narrower, as the distance from the growth cartilage increases, facts which may be connected with the formation of the new compact bone, on inner and outer surfaces, described above.

SUMMARY

1. After fracture, or attempted fracture, of the fibula, guinea-pigs on normal diets showed formation of new bone on the surfaces of the diaphyses of both tibia and fibula of the operated leg.

2. Guinea-pigs which were made partially deficient in vitamin C either before, or after, the operation, showed similar but often enormously larger thickenings.

3. In dietetically normal animals the thickenings never developed except after operation, and in partially deficient animals the thickenings occurred more frequently when the operation had been performed, but also occurred when there had been no operation.

4. The porosis of scurvy was seen in the old bone of the original diaphyseal wall but not in the new bone of the thickenings.

5. In both dietetically normal, and partially deficient animals, the new bone was trabecular in structure, not compact like the old diaphyseal bone. The trabeculae radiated from the surface of the bone outwards to the fibrous layer of the periosteum. Since their extent was much greater longitudinally than circumferentially, they were really narrow and irregular longitudinal ridges.

6. The thickenings developed in a widened periosteal cambium which formed when the fibrous layer of the periosteum had been separated from the bone beneath it by the post-operative oedema, by direct mechanical action in the operation itself, or by muscular action. In the partially deficient animals, the greater thickness of the new bone reflected the easier detachment of the periosteum and its less ready re-attachment, a greater tendency to superficial resorption of old bone, and the greater and more lasting oedema caused by the injury, than occurred in animals on normal diets.

7. The thickenings were sometimes well vascularized by vessels between the trabeculae, but more often were almost avascular. This condition of the new bone was associated with, and probably caused by, the avascular state of the hyperplastic connective tissue which developed in place of the degenerate musculature (see the first paper of this series). The thickenings which developed in dietetically normal animals after operation were quickly supplied with vessels, but in the partially vitamin C-deficient animals the new bone, like the new connective tissue, usually did not become vascularized, or only very poorly so. A richly vascular bed is not a necessary condition of bone formation.

8. The factors determining the formation of compact or trabeculae bone, and of the radiating architecture of the new bone, are discussed. When osteoblasts were scattered through a wide cambial tissue, trabecular bone was formed, but when a thin layer of osteoblasts was held against the old bone by a closely attached fibrous layer, the new bone was compact. The radiating architecture of the new bone was determined by a pre-existing radial arrangement of the collagen fibres in the widened cambium. It is argued that the orientation of the collagen fibres was determined by tensions, set up by the oedema, between the surface of the old bone and the fibrous layer of the periosteum.

9. When animals which had been kept for long periods on diets partially deficient in vitamin C, and which had developed trabecular thickenings, were given ample supplies of the vitamin, the addition of new bone to the surfaces of the trabeculae converted the trabecular bone into compact bone, while resorption from both periosteal and endosteal surfaces of the bone reduced its thickness. The reduction in thickness by superficial resorption was complicated by the deposition of new compact circumferential bone, whose formation may have been connected with form changes normally associated with growth.

REFERENCES

Baetjer, F. H. (1919). The roentgenologic signs of joint lesions in children. *Med. Clin. N. Amer.* **2**, 1703–1714.

Bromer, R. S. (1928). The roentgen-ray diagnosis of infantile scurvy. *Amer. J. Roentgenol.* **19**, 112–125.

Bromer, R. S. (1943). A critical analysis of the roentgen signs of infantile scurvy. *Amer. J. Roentgenol.* **49**, 575–579.

Caffey, J. (1946). Infantile cortical hyperostoses. *J. Pediat.* **29**, 541–559.

Chiari, O. von (1938). Über Skeletterkrankungen im Kindesalter im Röntgenbild mit besonderer Berücksichtigung des Säuglingsalters. *Wien. klin. Wschr.* **51**, 1264–1266.

Ellis, R. W. B. (1938–9). Vitamin-C deficiency and periostitis of both ulnae. Scurvy. *Proc. R. Soc. Med.* **32**, 139–141.

Follis, R. H. Jr. (1943). Effect of mechanical force on skeletal lesions in acute scurvy in guinea-pigs. *Arch. Path. Lab. Med.* **35**, 579–582.

Haldeman, K. O. (1932). The role of periosteum in the healing of fractures; an experimental study *Arch. Surg., Chicago*, **24**, 440–450.

Hart, C. & Lessing, O. (1913). *Der Skorbut der kleinen Kinder.* Enke, Stuttgart.

Hertz, J. (1936). *Studies on the Healing of Fractures with Special Reference to the Significance of the Vitamin Content of the Diet.* Oxford Univ. Press.

Höjer, J. A. (1924). Studies in scurvy. *Acta paediatr., Stockh.*, **3**, Suppl.

Koch, H. (1924). Experimentelle Studien über Knochenregeneration und Knochencallusbildung. *Beitr. klin. Chir.* **132**, 364–440.

Kodicek, E. & Murray, P. D. F. (1943). Influence of prolonged partial deficiency of vitamin C on the recovery of guinea-pigs from injury to bones and muscles *Nature, Lond.*, **151**, 395–396.

LANDAUER, W. (1927). Untersuchungen über Chondrodystrophie. 1. Allgemeine Erscheinungen und Skelett chondrodystrophischer Hühnerembryonen. *Roux Arch. Entw. Mech. Organ.* **110**, 195–278.

LEVY, O. (1904). Über den Einflüss von Zug auf die Bildung faserigen Bindegewebes. *Arch. Entw. Mech. Org.* **18**, 184–245.

LEXER, E. (1922). Über die Entstehung von Pseudarthrosen nach Frakturen und nach Knochentransplantationen. *Arch. klin. Chir.* **119**, 520–607.

MEYER, A. W. & MCCORMICK, L. M. (1928). Studies in scurvy. *Stanford Univ. Publ.* (c), **2**, no. 2, 1–107.

MOURIQUAND, G., TÊTE, H., WENGER, G. & VIENNOIS, P. (1937). Syndrome rhumatisme ankylosant dans l'avitaminose C partielle. *C.R. Acad. Sci., Paris*, **204**, 921–922.

MOURIQUAND, G., & DAUVERGNE, M. (1938). Ostéopathies par carence. Évolution des ostéoses et périostéoses par avitaminose C chronique. *Pr. méd.* **46**, 1081–1082.

MOURIQUAND, G., DAUVERGNE, M. & EDEL, V. (1940). Ostéopathies par carence. Décalcification irréversible du col fémoral dans l'avitaminose C chronique. *Pr. méd.* **48**, 268–269.

MOURIQUAND, G., DAUVERGNE, M., TÊTE, H., & EDEL, V. (1940). Ostéophytes par avitaminose C chronique. *C.R. Acad. Sci., Paris*, **210**, 515–516.

MURRAY, P. D. F. (1926). An experimental study of the development of the limbs of the chick. *Proc. Linn. Soc. N.S.W.* **51**, 187–263.

MURRAY, P. D. F. (1936). *Bones*, pp. 1–203. Cambridge Univ. Press.

MURRAY, P. D. F. & KODICEK, E. (1946). Some histological effects of partial deficiency of vitamin C on healing processes: the influence on bone repair. *Proc. nutrit. Soc.* **4**, 200–203.

MURRAY, P. D. F. & KODICEK, E. (1949). Bones, muscles and vitamin C. 1. The effect of a partial deficiency of vitamin C on the repair of bone and muscle in guinea-pigs. *J. Anat., Lond.*, **83**, 158.

MURRAY, P. D. F. & SELBY, D. (1930). Intrinsic and extrinsic factors in the primary development of the skeleton. *Roux Arch. Entw. Mech. Organ.* **122**, 629–662.

SCOTT, W. C. (1941). Epiphyseal dislocations in scurvy. *J. Bone Jt Surg.* **23**, 314–322.

STUDITSKI, A. (1934). The mechanism of the formation of regulating structures in the embryonic skeleton. *C.R. Acad. Sci. U.R.S.S.* N.S. **4**, 637–640.

WEIDENREICH, F. (1923). Knochenstudien. 1. Über Aufbau und Entwicklung des Knochens und den Charakter des Knochengewebes. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **69**, 382–466.

WEIDENREICH, F. (1930). Das Knochengewebe. In v. Möllendorff's *Handbuch der mikroscopischen Anatomie des Menschen*, **2**, zweiter Teil, 391–520.

WEISS, P. (1928). Experimentelle Organisierung des Gewebewachstums *in vitro*. *Biol. Zbl.* **48**, 551–566.

WEISS, P. (1929). Erzwingung elementarer Strukturverschiedenheiten am *in vitro* waschsenden Gewebe. Die Wirkung mechanischer Spannung auf Richtung und Intensität des Gewebewachstums und ihre Analyse. *Roux Arch. Entw. Mech. Organ.* **116**, 439–554.

WEISS, P. (1933). Functional adaptation and the role of ground substances in development. *Amer. Nat.* **67**, 322–340.

WIMPERGER, H. (1935). Klinisch-radiologische Diagnostik von Rachitis, Skorbut, und Lues congenita im Kindesalter. *Ergebn. inn. Med. Kinderheilk.* **28**, 264–370.

WURMBACH, H. (1928). Histologische Untersuchungen über die Heilung von Knochenbrüchen bei Säugern. *Z. wiss. Zool.* **132**, 200–256.

ABBREVIATIONS

camb.	cambial zone of periosteum	*haem.*	haemorrhage
circ.lam.	circumferential lamellae	*mar.*	marrow
con.th.	consolidated thickening	*n.b.*	new bone
fib.	fibula	*o.b.*	old bone
fib.b.	fibrillar bone	*peri.*	periosteum
fib.per.	fibrous layer of periosteum	*por.*	porosis cavity
gran.b.	granular bone	*shell.*	circumferential bony shell

Plate 1

Fig. 1. Normal guinea-pig. Development of subperiosteal thickening on lateral wall of tibia after fracture of fibula. A, day of fracture; B, 19th day; C, 50th day after fracture.

Fig. 2. Guinea-pig at first on normal diet, later on partially vitamin C-deficient diet. Left tibia and fibula. From Exp. 4, group 4. A, day of operation, normal diet; B, 15th day after operation, 1st day of partially deficient diet; C, 28th day after operation, 14th day of partially deficient diet; D, 81st day after operation, 67th day of partially deficient diet. Tibial and fibular thickenings, at first slight, become greatly enlarged after prolonged partially deficient diet.

Fig. 3. Normal guinea-pig. Transverse section of normal tibia. Haematoxylin and eosin. ×15.

Fig. 4. Normal guinea-pig. Part of longitudinal section of normal tibia. Azan. ×15.

Fig. 5. Normal guinea-pig. Part of longitudinal section of diaphyseal wall of tibia, showing new trabecular subperiosteal bone, 16th day after fracture of fibula. Haematoxylin and eosin. ×41.

Fig. 6. Normal guinea-pig. Part of longitudinal section of diaphyseal wall of tibia, 3rd day after fracture of fibula. Fibrous layer of periosteum lifted from bone by haemorrhage. Haematoxylin and eosin. ×195.

Fig. 7. Normal guinea-pig. Part of longitudinal section of diaphyseal wall of tibia, 4th day after fracture of fibula. Space beneath fibrous layer of periosteum filled by cells. Azan. ×195.

Plate 2

Fig. 8. Normal guinea-pig. Part of longitudinal section of diaphyseal wall of tibia, 5th day after fracture of fibula. Formation of new trabecular bone in the widened cambium. Haematoxylin and eosin. ×195.

Fig. 9. Partially vitamin C-deficient guinea-pig. Transverse section of tibia showing great subperiosteal thickening, and porosis of the old bone. Haematoxylin and eosin. ×9·5. From Exp. 4, group 4, 81st day of experiment, 67th day of partially deficient diet.

Fig. 10. Partially vitamin C-deficient guinea-pig. Transverse section of fibula showing subperiosteal thickening. Haematoxylin and eosin. ×31. From Exp. 3, group 3, 101st day of experiment, 86th day of partially deficient diet.

Fig. 11. Partially vitamin C-deficient guinea-pig. Transverse section of tibia and fibula, to show the two types of architecture (compact old bone, partly eroded by porosis, and trabecular new bone) separated by a darkly staining cement line. Haematoxylin and eosin. ×17. From Exp. 3, group 3, same animal as Fig. 10.

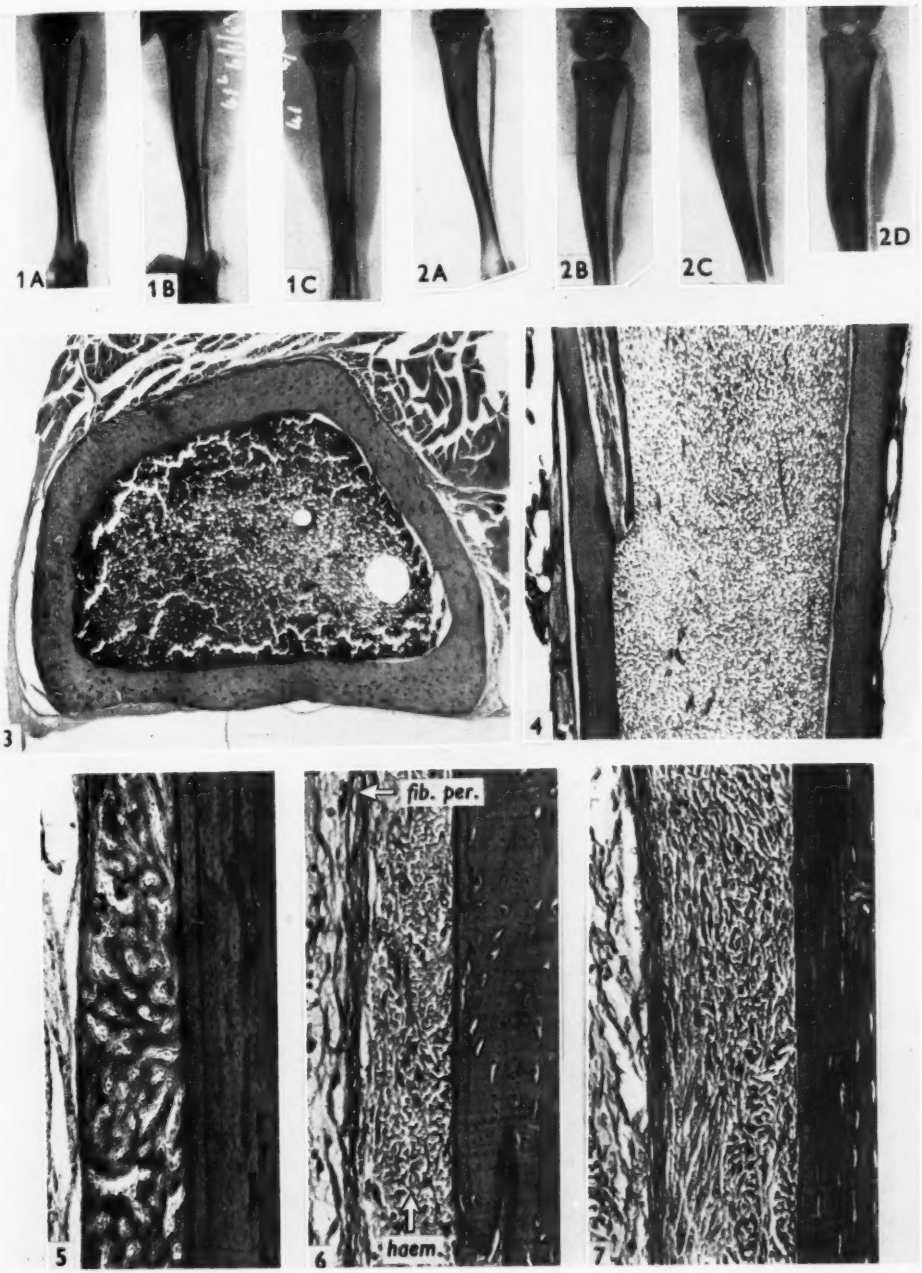
Fig. 12. Partially vitamin C-deficient guinea-pig. Part of a tangential section of the tibial wall, passing through the new trabeculae, showing that what appear in transverse sections to be radial spokes are really narrow ridges. Haematoxylin and eosin. ×48. From the same specimen as Fig. 9.

Plate 3

Fig. 13. Partially vitamin C-deficient guinea-pig. Part of a longitudinal section of the tibia, showing the diaphyseal wall composed entirely of new trabecular bone with a paper-thin zone of old compact bone remaining (bordering the marrow cavity, below). Haematoxylin and eosin. ×65. From Exp. 6, group 4, 85th day after beginning the experimental diet, and 62nd day after the operation.

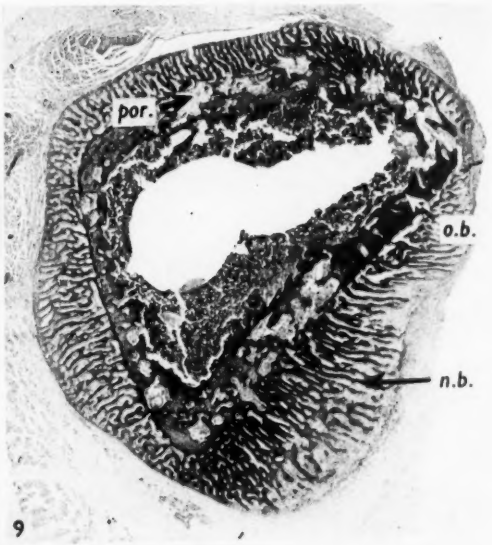
Fig. 14. Partially vitamin C-deficient guinea-pig. Part of a transverse section of the tibia, showing the radiating trabeculae of the thickening joined at the surface by a thin shell of circumferential bone. Haematoxylin and eosin. ×74. From Exp. 3, group 3, the same animal as Figs. 10 and 11.

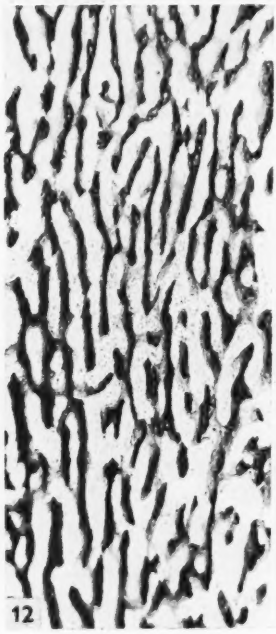
Fig. 15. Partially vitamin C-deficient guinea-pig. An area of the subperiosteal thickening showing granular bone; notice the large lacunae and granular matrix. Haematoxylin and eosin. ×940. From Exp. 3, group 3, 101st day after operation, 86th day after beginning the experimental diets.



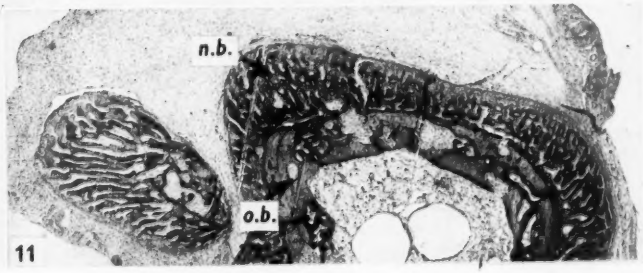
MURRAY AND KODICEK—BONES, MUSCLES AND VITAMIN C

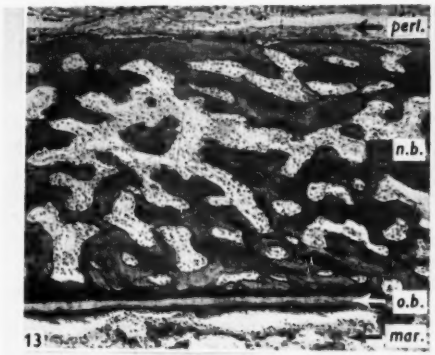
fib.
o.b.
n.b.
8


por.
o.b.
n.b.
9

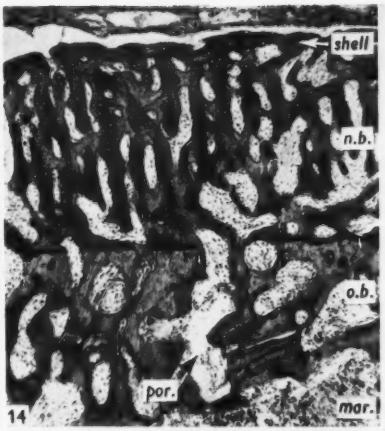

12

n.b.
o.b.
10

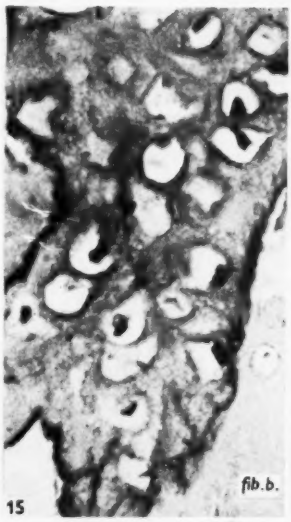

n.b.
o.b.
11



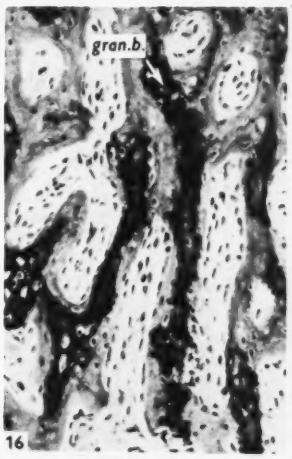
13



14

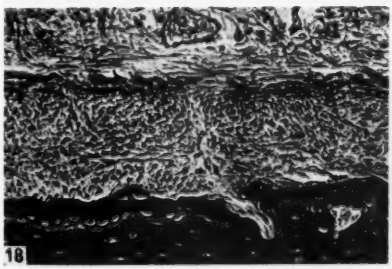


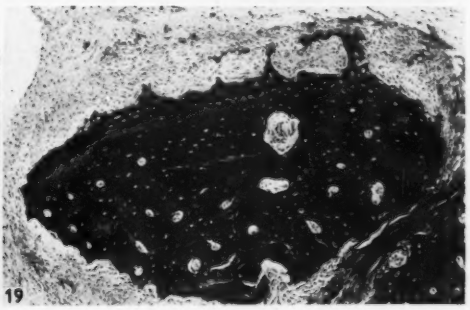
15

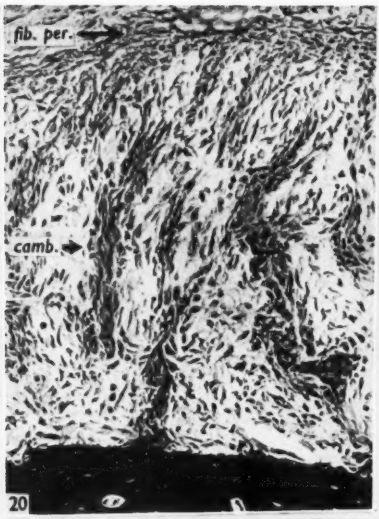


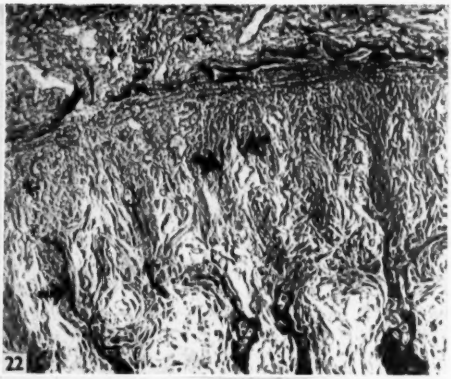
16

17


18

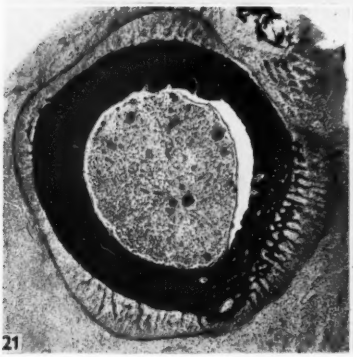

19


fib. per.
camb.
20


22

circ. lam.
con. th.
23
circ. lam.

por.
24


21

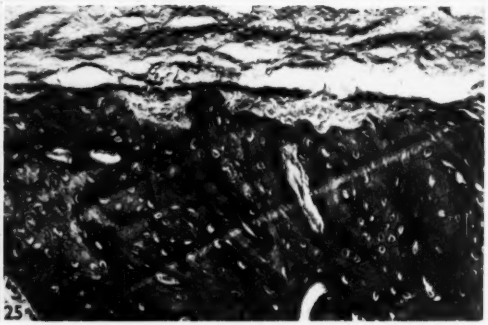

25

Fig. 16. Partially vitamin C-deficient guinea-pig. Part of a tangential section of the tibial wall, passing through new trabeculae, showing granular (dark) and fibrillar (pale) bone. Haematoxylin and eosin. ×185. From the same specimen as Fig. 9.

Fig. 17. Partially vitamin C-deficient guinea-pig. Part of a trabecula of new bone in a subperiosteal thickening, impregnated with silver by Wilder's method and stained with haematoxylin. The granular bone at the core of the trabecula is almost without fibres, while the fibrillar bone covering its surface is densely fibrous. × 940. From Exp. 3, group 3, the same animal as Fig. 10.

Fig. 18. Partially vitamin C-deficient guinea-pig. Part of a transverse section of the tibia, showing the fibrous layer lifted from the bone and the space beneath filled by an enlarged cambial tissue. Azan. ×195. From Exp. 9, 6th day after operation, 31st day after beginning the experimental diet.

Fig. 19. Partially vitamin C-deficient guinea-pig. Transverse section of fibula, showing evidence of superficial resorption of the bone, and a widened cambial tissue. Azan. ×90. From Exp. 9, the same animal as Fig. 18.

PLATE 4

Fig. 20. Partially vitamin C-deficient guinea-pig. Part of a transverse section of the tibia showing an early stage in the development of bone in the widened cambial tissue, with groups of osteoblasts radially arranged. Azan. ×195. From Exp. 9, 12th day after operation and 37th day after beginning experimental diets.

Fig. 21. Partially vitamin C-deficient guinea-pig. Transverse section of tibia. Widened cambium and developing trabeculae of new bone, radially orientated. Azan. ×20. From Exp. 9, the same animal as Fig. 20.

Fig. 22. Partially vitamin C-deficient guinea-pig. Part of a transverse section of the tibia, showing the peripheral part of the widened cambial tissue. Below, the ends of new bony trabeculae; these are continued to the fibrous layer of the periosteum by strands of collagen fibres (indicated by arrows). ×195. From Exp. 9, the same animal as Fig. 20.

Fig. 23. Part of the tibial wall of a guinea-pig which, after prolonged partial vitamin C-deficiency, was cured with ample vitamin C. The section shows consolidation of the subperiosteal thickening and new internal and peripheral circumferential lamellae. Haematoxylin and eosin. ×135. From Exp. 7, group 2, 107th day of experiment, 41st day after return to balanced diet; no operation.

Fig. 24. Part of the tibial wall of the other leg of the same animal as Fig. 23. Consolidation of the thickening, new internal circumferential lamellae, and cavities in the thickening formed by resorption. Haematoxylin and eosin. ×92.

Fig. 25. Part of the tibial wall of the same animal as in Figs. 23 and 24. Evidence of resorption from the surface of the thickening. Haematoxylin and eosin. ×200.

AN UNUSUAL ANOMALY OF M. FLEXOR DIGITORUM LONGUS

By T. E. BARLOW
Department of Anatomy, University of Durham, Newcastle upon Tyne

The anomaly occurred in a male subject, aged 71 years, and was bilateral, except for slight differences. The condition on the left side is described first. The tendon of the flexor digitorum longus was normal until it reached the foot. It then divided at the usual point, but into three tendons only. Those to the 2nd and 3rd toes were normal but the tendon to the 4th toe was a small slip forming not more than a quarter of the total number of tendinous fibres reaching that digit; the remainder of the fibres passing to the 4th toe, as well as those to the 5th toe, being derived from an anomalous flexor accessorius.

Flexor digitorum accessorius (quadratus plantae) was composed of three parts, the most superficial of which was a small muscle arising by two heads, a tendinous one from the medial tubercle of the calcaneum just medial to the long plantar ligament and a muscular one from the sheath of flexor hallucis longus and flexor digitorum longus. It joined the lateral side of a long tendon inserted into the terminal phalanx of the little toe and contributed also to the tendon of the 4th toe.

The intermediate part of the muscle was the main source of the tendons to the 4th and 5th toes: it was a triangular sheet arising by fleshy fibres from the under surface of the calcaneum behind the sustentaculum tali as far back as the medial tubercle. Its tendon of insertion split to go to both the 4th and 5th toes; that to the 5th being joined by the fleshy fibres, already mentioned, from the superficial part of the muscle.

The deep part of the muscle arose from the underside of the calcaneum in front of and lateral to the sustentaculum tali and was inserted in the normal manner of the flexor accessorius into the tendon of flexor digitorum longus. The 4th lumbrical arose from the tendons to the 4th and 5th toes.

In the right foot the same condition was found except that the tendon to the 4th toe was derived equally from flexor digitorum longus and accessorius.

The interest of this case lies in the support it gives to the phylogenetic history of the flexor muscles of the toes as interpreted by Wood Jones (1944, pp. 219–225).

The underlying factor in this phylogeny is the necessity for the digital flexors to remain contracted, while the extensors also contract, in order to enable the toes of the supporting and propelling foot to push against the ground at the moment of take off in walking. While the flexed toes are pushing against the ground the digital extensors have to act from them to pull the leg forwards over the ankle joint.

The necessary concomitant of extensor contraction is relaxation of the belly of the long flexor, but at the same time the toes must remain flexed against the ground. This conflict of interests has been solved by turning the superficial flexor into an intrinsic muscle of the foot by divorcing it from its belly in the leg (plantaris) and by transferring the work of the deep flexor in this connexion to the flexor accessorius.

The logical conclusion of this story would be to divorce the deep flexor also from its belly in the leg, and the initial stage in such a divorcement appears to be occurring in the present case.

The function of flexor accessorius is often said to be to draw the tendons into which it is inserted into a less oblique position.

In this connexion it is interesting to note that in the present instance the anomalous tendons to the 4th and 5th toes having separated themselves from the remaining

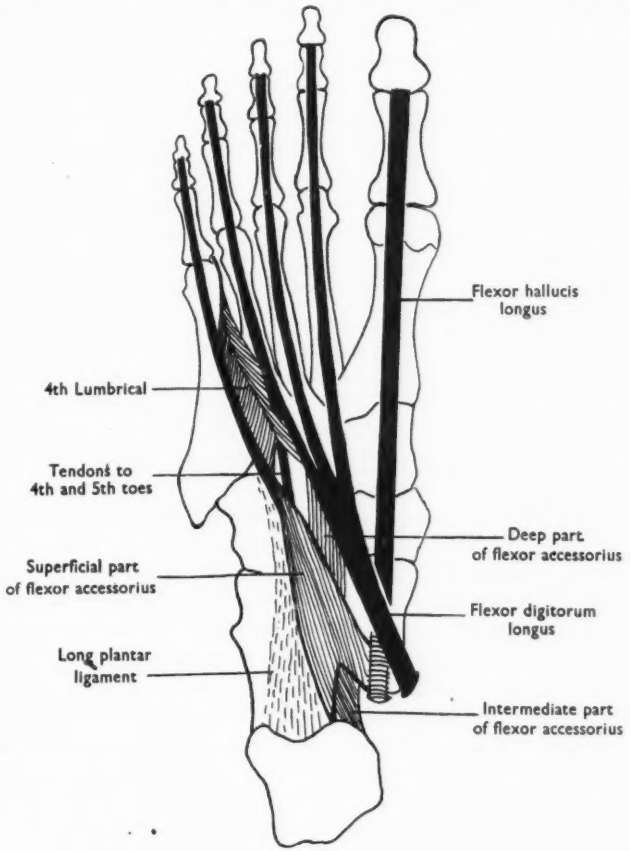

Flexor hallucis longus
4th Lumbrical
Tendons to 4th and 5th toes
Deep part of flexor accessorius
Superficial part of flexor accessorius
Flexor digitorum longus
Long plantar ligament
Intermediate part of flexor accessorius

Fig. 1.

tendons of flexor digitorum longus, not only maintain their original oblique direction but, having obtained a bony attachment to the medial tubercle of the calcaneum, they discard any further assistance from the flexor accessorius, for the lateral head of that muscle is absent.

Here, then, is a situation that should help to dispel the idea that living muscular tissue is used instead of the usual fibrous pulley to change the direction of a tendon or group of tendons.

SUMMARY

An interesting anomaly of flexor digitorum longus is described and a reason for its presence adduced. A further reason is given for supposing that flexor accessorius (quadratus plantae) does not perform the functions usually ascribed to it.

REFERENCE

JONES, F. WOOD (1944). *Structure and Function as seen in the Foot.* London: Baillière, Tindall and Cox.

ALKALINE PHOSPHATASE ACTIVITY IN NORMAL AND DEGENERATED PERIPHERAL NERVES OF THE RABBIT

By JUNE MARCHANT, *Department of Embryology, University College, London**

INTRODUCTION

The process of nerve degeneration has many features in common with the healing of skin wounds; both involve cell proliferation and migration and the laying down of collagen fibres. Fell & Danielli (1943) using the histochemical technique of Gomöri (1939) and Takamatsu (1939), demonstrated alkaline phosphatase activity in healing skin wounds and burns of rats greatly in excess of that found in normal skin. The increased amount of enzyme was situated on invading polymorphs, cell nuclei and newly formed collagen fibres. They suggested that the enzyme associated with the collagen fibres might take part in the 'metabolic processes more intimately concerned in the laying down of collagen', and further support was given to this hypothesis by their more recent work (Danielli, Fell & Kodicek, 1945), which showed that the poor differentiation of collagen fibres in healing skin wounds of scorbutic guinea-pigs was associated with an absence of histochemically demonstrable phosphatase.

Degenerating peripheral nerve provides useful material for testing further this hypothesis of Fell & Danielli. Degenerative processes take place almost uniformly throughout the peripheral stump of such a nerve, which forms a large and clearly defined mass of uniform material for investigation. A considerable amount of collagen is formed during degeneration and the time-course of its formation is better known than in skin, for it has been studied quantitatively by Abercrombie & Johnson (1946*a*). They found, by chemical methods, that the amount of collagen in the degenerating rabbit sciatic nerve increased throughout a period of at least 200 days after the nerve was severed. Furthermore, collagen formation in this tissue is largely dissociated from cell-proliferation, which ceases after 25 days of degeneration (Abercrombie & Johnson, 1946*b*); whereas in skin, so far as present information goes, the two processes largely overlap in time and consequently may confuse interpretation of the associated phosphatase activity. Another convenient feature of degenerating nerve lies in the fact that, except in the very localized region of actual trauma, there is no invasion of the tissue by polymorphs, which might complicate the issue since they are very rich in phosphatase.

If Fell & Danielli are correct in their hypothesis that alkaline phosphatase is concerned in the laying down of new collagen, we may expect to find this enzyme present in degenerating peripheral nerve throughout the prolonged period in which, according to Abercrombie & Johnson, collagen formation occurs.

Studies of alkaline phosphatase in degenerated peripheral nerve do not appear to have been undertaken hitherto. Landow, Kabat & Newman (1942) were unable to demonstrate histochemically the presence of this enzyme in the undegenerated sciatic nerves of cat, mouse and man, exept in the vascular endothelium; in the chicken the

* Now with the Biophysics Research Unit, King's College, London.

Schwann sheath showed a decided reaction, though there was none in axons, or the myelin sheath.

MATERIAL AND METHOD

The material used for this work was obtained from adult rabbits. Several kinds of undegenerated nerves were studied, namely the sciatic (peroneal and tibial divisions) anterior mesenteric, greater splanchnic and vagus nerves. Degenerated material was provided by the peripheral stump of the severed sciatic nerve. The nerve had previously been cut high up in the thigh under nembutal and ether anaesthesia and the peripheral stump allowed to degenerate for a period of time varying from 2 to 100 days, the rabbit being then killed by air embolism. To prevent re-innervation of the peripheral stump during the longer periods of degeneration (from 50 days onwards) the central stump of the nerve was sutured to the undersurface of the skin. In experiments involving degeneration for 25 days, or less, re-innervation was prevented by removing about 1 cm. of the nerve. Both sciatic nerves of each rabbit were usually severed, but on different occasions, so that at autopsy each animal provided nerves of two different periods of degeneration. In some cases pieces of undegenerated nerve removed at the operation were used for comparison with degenerated nerve from the same rabbit taken at autopsy.

Pieces of nerve 0·5–1·0 cm. long were fixed in 80 or 95 % alcohol, fixation in 80 % for about 20 hr. giving tissue of the best cutting quality. Some of these pieces were then taken down to water, and frozen sections were cut from them; other pieces were dehydrated, cleared in benzene, embedded in paraffin at 56° C., and longitudinal and transverse sections of each piece were then cut at a thickness of 7 μ. Sections were treated by Danielli's (1946) modification of Gomöri's technique, being incubated for 18–22 hr. at 37° C. and pH 9·3 with sodium glycerophosphate in the presence of calcium ions and activating magnesium ions. By this method a precipitate of calcium phosphate is formed in the vicinity of the enzyme, and this is rendered visible by conversion to black cobaltous sulphide.

A few experiments were made to ensure the validity of the technique in its application to the material used in this investigation. From each of four undegenerated sciatic nerves one slide was given the full Gomöri technique; a second received no activating magnesium ions in the incubating mixture, and a third received no glycerophosphate; a fourth slide was incubated in the normal mixture with the addition of 0·1 % potassium cyanide (known to inhibit alkaline phosphatase; Massart & Dufait, 1942), and a fifth slide was heated to 90° C. in water for 15 min., prior to normal treatment, in order to destroy the enzyme (Danielli, 1946). The sections on the first slide showed some blackening, those on the second showed less, and the remainder showed none. Similar experiments carried out on the other undegenerated nerves used in this study gave identical results. It is concluded that in peripheral nerve the technique reveals the activity of alkaline phosphatase as it has been shown to do in other tissues.

Control sections of nerve, omitting glycerophosphate from the substrate, were incubated as a routine in order to reveal any phosphate already present. Sections of kidney (known to be rich in phosphatase) were prepared together with those of the nerves in order to ensure that the technique was working satisfactorily. A further

check was provided by polymorphs in the blood vessels of the nerve, which appear intensely black after the phosphatase technique has been applied to them. Only sections in which polymorphs were intensely stained will be considered.

The location of phosphatase in relation to collagen was investigated by comparison of sections stained for the enzyme with sections fixed in 4 % formol-saline and stained by Gomöri's (1937) silver-impregnation method for collagen and reticulin. For general histological comparison, sections fixed in Susa's fixative and treated with Masson's light-green method were used. For revealing axons in undegenerated and short-term degenerated nerves, Bodian's (1936) silver method was used.

RESULTS

Controls

Control sections of undegenerated nerves, and sections of nerves degenerated for all the periods of time employed in this work, did not in any case show any blackening when the Gomöri technique with the omission of glycerophosphate was applied to them. Pre-existing phosphate was therefore negligible.

Undegenerated peroneal and tibial nerves

The greater part of the undegenerated nerve showed little positive reaction, but a number of sporadically distributed intense black 'streaks' were often visible in longitudinal paraffin sections (Pl. 1, fig. 1). When the number of 'streaks' in a section was large and their intensity of staining great, other structures in the nerve were often stained, particularly the precipitated myelin framework and the axons of myelinated fibres, and the intensity of staining of these structures was generally greater in regions in close proximity to the 'streaks'. In transverse sections (Pl. 1, fig. 2) the 'streaks' were apparently situated in interstices between the myelinated fibres.

The above description is based on eleven sciatic nerves. Considerable variation occurred, however, in the amount of blackening in these nerves. In some the 'streaks' were numerous, but in two nerves none was visible, though polymorphs in blood vessels were stained.

Other undegenerated nerves

The distribution of alkaline phosphatase activity in several other undegenerated nerves, differing in their distributions of fibre sizes, was investigated in an attempt to analyse further the curious distribution in the peroneal and tibial nerves.

Vagus, depressor branch. This nerve contains, like the peroneal nerve, a large number of heavily myelinated fibres. Like the latter, its alkaline phosphatase was distributed mainly in several 'streaks', with a few nuclei and some myelin framework also stained. (Three nerves.)

Greater splanchnic nerve. Most of the fibres of this nerve are finely myelinated, with a very small number of heavily myelinated fibres among them. There were a few 'streaks' of intense alkaline phosphatase activity, lesser activity in nuclei and axons of all myelinated fibres, and slight activity in myelin framework. (Three nerves.)

Anterior mesenteric nerve. This unmyelinated nerve was usually obtained with its

ganglion. Throughout the nerve, nuclei were strongly stained and also fine fibres of uncertain nature (Pl. 1, fig. 3). In some of these nerves the intensity of staining diminished with increasing distance from the ganglion. The ganglion cells showed intense phosphatase activity in the nucleoli and nuclear membrane, but not in the cytoplasm. All the tissue surrounding the ganglion cells was very intensely stained (Pl. 1, fig. 4). (Three nerves.)

Vagus, gastric branch. The whole of this unmyelinated nerve showed a fairly intense phosphatase activity associated with all the nuclei, and with fine fibres, as in the anterior mesenteric nerves. There was no localization in 'streaks'. (Three nerves.)

Degenerated sciatic nerve

The most proximal piece of the peripheral stump, comprising 2 or 3 mm. of nerve, was affected by the trauma of operation, and is considered, together with any scar tissue which attached the end of the nerve to the adjacent muscle, as the 'traumatized' region. Other pieces are referred to as the 'peripheral' region.

Two days of degeneration, peripheral region. The alkaline phosphatase reaction was similar to that of the undegenerated nerve. Some frozen sections showed the 'streaks' particularly clearly. Each 'streak' was fibrous, and contained blackened nuclei (Pl. 2, fig. 5). Some could be traced for 1 mm. or more (Pl. 2, fig. 6). (Four nerves.)

Traumatized region. This was like the peripheral region, but myelin remains and axons were stained rather more at the traumatized end of the nerve, which was surrounded by many intensely staining polymorphs. These had not invaded the nerve (Pl. 2, fig. 6). (Four nerves.)

Five days of degeneration, peripheral region. There had been little change since 2 days except that more nuclei showed a phosphatase reaction, and the surrounding cytoplasm sometimes showed a slight reaction also. (Three nerves.)

Traumatized region. The cut end of the peripheral stump of the nerve was now securely bound to the adjacent muscle by connective tissue. In silver-impregnated sections this was seen to consist of a tangle of fibres of all sizes which appeared to lie in a matrix. Some were wavy purple ribbons, others were tangles of fine black threads, but most were very fine threads which were not intensely argyrophil. When the alkaline phosphatase technique was applied to this connective tissue there was no blackening of any fibres. Polymorphs had usually completely disappeared. Just beyond the cut ends of about half the nerves of this group a few nuclei in the connective tissue were intensely stained for phosphatase. (Eight nerves.)

Ten days of degeneration, peripheral region. A positive phosphatase reaction occurred in the great majority of nuclei of all kinds of cells, in certain fine longitudinally running fibrils (believed to be mainly Schwann cytoplasm) and in myelin debris. In some nerves the distribution of phosphatase in longitudinal sections was streaky (Pl. 2, fig. 8) as at earlier stages, though the intensity of staining of the 'streaks' and their contrast with the rest of the nerve was not usually so great. The 'streaks' consisted of groups of small Schwann tubes (devoid of myelin debris) in which numerous fibrils and nuclei were particularly strongly stained (Pl. 2, fig. 7). In most of the nerves there were no 'streaks'. (Seven nerves.)

Traumatized region. The amount of connective tissue binding the end of the nerve to the muscle had increased, and in silver-impregnated sections many more collagen

fibres than at 5 days were visible, orientated rather more in a direction parallel with the nerve than otherwise. Blood vessels were easily distinguishable by their black reticular coats. There was no positive phosphatase reaction in the binding connective tissue except in the nuclei of blood-vessel walls and of polymorphs, and in some nerves, in a few nuclei just beyond the cut ends of the nerve fibres. The connective tissue fibres were completely negative. (Pl. 2, fig. 8 and Pl. 3, fig. 9). (Seven nerves.)

Fifteen days of degeneration, peripheral region. Some streakiness of staining was still occasionally discernible. Almost all the Schwann, endoneurial and blood-vessel nuclei were strongly stained in most nerves (Pl. 3, fig. 10), and nuclei of perineurial cells were sometimes slightly stained. In some cells the cytoplasm of endoneurial cells could be seen to be feebly positive. In contrast to the 10-day nerves, stained myelin debris was almost entirely absent (Pl. 3, fig. 10). The blackened fibrils, present in the 10-day nerves, were more numerous, at any rate in the larger tubes, and often thicker. In transverse sections they appeared as rings of small dots against the inside of the neurilemma (i.e. the membrane lining the Schwann tube) and projecting into the lumen of the tube or sometimes apparently embedded in the substance of the neurilemma (Pl. 3, fig. 11). In longitudinal section some of the fibrils could be seen to end in perinuclear cytoplasm, identifying them as parts of Schwann cells (Pl. 3, fig. 10). In longitudinal sections stained with silver or Masson's light green there were rather more longitudinally orientated fibres present than at 10 days of degeneration, suggesting that new collagen had started to form. (Seven nerves.)

Traumatized region. Silver impregnation showed the connective tissue binding the end of the peripheral stump to the muscle to be deeply packed with thick, wavy parallel ribbons of collagen, all more or less orientated in the same direction as the nerve. This tissue showed little change in phosphatase reaction from the same region after 10 days of degeneration, though rather more nuclei of cells were stained, and their cytoplasm also showed a slight reaction. (Six nerves.)

Twenty-five days of degeneration, peripheral region. Signs of collagen formation in the form of an increased number of fine fibrils in the Schwann tube walls were quite obvious in silver-impregnated sections. The phosphatase reaction was little changed since 15 days of degeneration, except that the phosphatase-positive fibrils were thicker and more numerous. (Eight nerves.)

Traumatized region. This was similar to the 15-day traumatized region. (Seven nerves.)

Fifty days of degeneration, peripheral region. There had been a great increase in the number of longitudinally running collagen fibres seen in the walls of the Schwann tubes in silver-impregnated sections. In sections stained for phosphatase, nuclei were still intensely stained and there had been a further increase in thickness of the blackened fibrils seen in longitudinal and transverse section. (Nine nerves.)

Traumatized region. In silver-impregnated sections it was impossible to distinguish the original site of the cut, for the Schwann tubes were very collagenized and were continuous with the collagen in the connective tissue binding the stump of the nerve to the muscle. Near the cut end of the nerve partitions of phosphatase-positive material could be seen within some of the Schwann tubes. Strands of cells with nuclei and cytoplasm stained for phosphatase were found amongst the binding connective tissue, having probably migrated out from the cut end of the nerve; and some

of these strands appeared to have invaded the epineurium, presumably by turning round and growing back along the nerve, as described by Masson (1932). (Eight nerves.)

One hundred days of degeneration, peripheral region. More extensive collagenization of the Schwann tube walls was obvious in silver-impregnated sections. In longitudinal sections stained for phosphatase nuclei were strongly positive, and very numerous fibrils were stained, some thicker ones intensely (Pl. 3, fig. 12). In transverse sections most of the staining other than that in nuclei was in the form of large lumps (fibrils seen end-wise) clearly situated within the lumina of the Schwann tubes. The main mass of new collagen, external to the neurilemma, was little stained (Pl. 4, fig. 13). (Three nerves.)

Traumatized region. In silver-impregnated sections no change other than more extensive collagenization was visible. Sections stained for phosphatase showed a very intense reaction in the strands of Schwann cells, which had wandered out from the cut ends of the Schwann tubes, and all surrounding tissue took up the stain to a certain extent (Pl. 4, figs. 14, 15). (Four nerves.)

Tissue cultures of degenerated sciatic nerve

It was considered that tissue culture studies of degenerated sciatic nerve might help in elucidating the nature of the phosphatase-positive fibres seen in sections. Accordingly, pieces of degenerated nerve were removed for culture under sterile conditions at autopsy. Cultures of the peroneal branch of 15 and 25 days-degenerated sciatic nerves in hanging drops of fowl plasma and embryo extract showed good outwandering of Schwann cells, fibroblasts and macrophages. It was found that, if the clots were made as thin as possible and the cultures washed for half an hour in saline at 37° C. prior to fixation in alcohol, the Gomöri alkaline phosphatase technique could be successfully applied without the cells being obscured by blackening of the clot. The explant appeared intensely black, and all types of cells that had wandered out were stained, nuclei fairly intensely, cytoplasm less so. The Schwann cell cytoplasm appeared extremely fibrous, and it was possible to trace these phosphatase-positive fibrils into the explant and show that they were continuous with the phosphatase-positive fibrils of the nerve, thus indicating that the latter were cytoplasm of Schwann cells. Controls incubated without glycerophosphate were unstained.

DISCUSSION

Reliability of the histochemical technique

This has been reviewed by Danielli (1946) and Lison (1948). The specificity of the reaction appears to be good provided adequate controls are done for comparison. A positive reaction reveals the presence of the enzyme, but a negative result does not necessarily indicate its entire absence, since part of the enzyme activity may be destroyed during the processes of fixation, embedding, etc. Diffusion of intermediary substances in the section does not seem to occur, according to these authors, since the same result can be obtained by rendering visible either the phosphate or the alcoholic end of the ester substrate. A weak point of the technique may, according to Lison (1948), lie in the possibility that the enzyme diffuses during fixation from

the exact sites where it occurred under physiological conditions. Certain of my observations seem to support the view that diffusion, presumably of the enzyme itself, occurs. In the undegenerated sciatic nerve, nuclei and the myelin framework may be stained by the Gomöri technique to an extent depending on the number and proximity of the intensely stained 'streaks'. In the 2-day degenerated nerve, these structures are more intensely stained toward the cut end of the nerve which bears a cap of intensely staining polymorphs. In the 100-day degenerated traumatized region, where the strands of Schwann cytoplasm that had wandered out sometimes show a very intense reaction, the surrounding connective tissue nuclei take up the stain to a certain extent, whereas, in sections of nerves where Schwann cytoplasm is not so intensely stained, connective tissue nuclei remain unstained. These and other observations suggest that, when a certain histological element is rich in phosphatase, some diffusion may occur, probably of the enzyme during fixation. The possibility of diffusion requires that no reliance should be placed on the finer points of localization, such as, for instance, the fact that all tubal and endoneurial nuclei, whatever their cell type, become phosphatase positive during degeneration. The existence of significant changes in distribution and activity of phosphatase during degeneration of the sciatic nerve is not called in question.

Alkaline phosphatase in undegenerated nerves

In the undegenerated sciatic nerves, except for two which were almost completely negative, phosphatase activity was chiefly localized in 'streaks'. These did not appear to involve the large fibres but to lie between them (Pl. 1, fig. 2). It was, however, difficult to decide from the undegenerated material what structures were stained in the 'streaks'. Very similar 'streaks' occurred during the early stages of degeneration (in some nerves up to 15 days), and here it seemed probable that they lay in the Schwann tubes formerly occupied by groups of small, perhaps unmyelinated, fibres. The hypothesis that the 'streaks' in undegenerated nerve represented groups of unmyelinated fibres and their associated Schwann cells cut somewhat obliquely led to the testing of other nerves. Those consisting mainly of unmyelinated fibres (gastric branch of the vagus, anterior mesenteric nerve) proved to be phosphatase-positive throughout. However, it must be admitted that this evidence is inconclusive since the fine details of the staining of these unmyelinated nerves were unlike the 'streaks' of the undegenerated sciatic, the unmyelinated nerves showing staining of numerous fine fibrils which were rarely distinguishable in the 'streaks'. An alternative hypothesis is, of course, that the 'streaks' are an artefact, due to diffusion and periodic precipitation of the enzyme during fixation, and peculiar to undegenerated nerves with large nerve fibres. On the basis of my data, however, no certain interpretation of the 'streaks' seems possible.

Alkaline phosphatase in the degenerated sciatic nerve

Between 5 and 10 days of degeneration the 'streaks' in the sciatic nerve disappeared, and from this time onwards phosphatase activity was uniform and increased progressively in amount, being chiefly associated with nuclei and with numerous fibrils. There is no doubt that many of these fibrils, probably all the intensely stained ones, consist of Schwann cell cytoplasm. This follows from their position within the

lumina of the Schwann tubes, which is especially evident after 50 or 100 days of degeneration. It is supported by the fact that in longitudinal section some fibrils could be traced up to the perinuclear cytoplasm of Schwann cells, and by the tissue culture results. Remains of fine axons, although persisting up to 10, 15 and occasionally 25 days of degeneration, judging from Bodian preparations, can obviously be dismissed as a basis for the phosphatase-positive fibrils in the nerves that have degenerated longer. A certain number of the fibrils, especially those apparently embedded in the substance of the tube wall, and the numerous very fine, weakly positive fibrils of the long degenerated nerves, may well be connective tissue fibres.

Besides the predominant staining of all nuclei and of Schwann cytoplasm in the degenerated sciatic nerves, endoneurial cytoplasm is also often stained. In view of the possibility of diffusion of the enzyme, it may be that phosphatase reaction in some cells does not signify the original distribution of the enzyme. But it is nevertheless clear that during Wallerian degeneration there is a real onset, and subsequent intensification, of widespread phosphatase activity in cells which before degeneration contained no demonstrable phosphatase (except in the 'streaks'). This activity is not associated with the wave of mitosis which occurs between 4 and 25 days of degeneration and which results in the steep rise of cell population during that time (Abercrombie & Johnson, 1946*b*), nor with the destruction of nerve fibres which occurs during the same period.

Fell & Danielli (1943) tentatively suggested that alkaline phosphatase localized on newly formed collagen fibres, played a part in the collagen formation of rat and guinea-pig skin scars. Later observations have shown that an increase in histochemically demonstrable alkaline phosphatase activity commonly seems to succeed trauma to a tissue, for instance in skin (Fisher & Glick, 1947), bone (Bourne, 1948), and liver (Mellors & Sugiura, 1948; Sherlock & Walshe, 1947; Sulkin & Gardner, 1948), and collagen formation also commonly succeeds trauma. But can the hypothesis of Fell & Danielli be generalized in the light of the present results on degenerated nerves? In the peripheral stump of degenerated rabbit nerves the period of time during which a widespread cellular alkaline phosphatase reaction occurred certainly coincided with the peculiarly long-drawn-out phase of collagen formation found by Abercrombie & Johnson (1946*a*); and the onset of this phosphatase reaction coincided with the earliest appearance of new collagen according to my silver preparation (at about 10 days of degeneration). This temporal relation is, however, the only evidence I found in my material suggesting that alkaline phosphatase may take part in the process of collagen formation, and by itself it is inconclusive. The reaction was not particularly localized on new collagen fibres, so that rabbit nerve differs in this from the rat and guinea-pig skin studied by Fell & Danielli. Furthermore, in the scar region of severed rabbit nerve there was no demonstrable localization of the enzyme on newly formed collagen fibres. In the scar region indeed the phosphatase reaction, apart from the initial polymorph reaction, was not conspicuous anywhere during the early stages after the operation, although collagen fibres apparently formed during this period, and with very much the same time relations as after skin injury (as described by Hunt, 1941). Only after about 50 days of degeneration did considerable blackening appear, and then in the strands of Schwann cells which had wandered out from the cut ends of the nerve fibres. The conclusion must be that the relation between

histochemically demonstrable alkaline phosphatase and collagen formation differs in different tissues; and a generalization of Fell & Danielli's hypothesis of a causal relation between the two cannot therefore be supported by the available evidence.

SUMMARY

1. Gomöri's histochemical technique for localizing alkaline phosphatase activity has been applied to various undegenerated nerves, and also to the degenerating peripheral stump of the sciatic nerve of rabbits.

2. Undegenerated nerves containing many myelinated fibres (sciatic, greater splanchnic and vagus depressor) showed phosphatase activity in sporadically distributed 'streaks', whose nature is discussed.

3. Undegenerated nerves containing mainly non-myelinated fibres (anterior mesenteric and vagus gastric) showed a widespread reaction in nuclei and in fine fibrils of uncertain nature.

4. In the degenerating peripheral stump of the cut sciatic nerve the 'streaks' had practically disappeared at the end of 10 days of degeneration, after which a reaction began to develop in nuclei of all kinds of cells and also in fibrils which mainly lined the Schwann tubes. Investigation of these fibrils by various methods, including tissue culture, indicated that most of them were cytoplasm of Schwann cells. They increased in size up to 100 days of degeneration at least, forming a thick, irregular lining to the neurilemma with intense phosphatase activity. The widespread enzyme activity in nuclei and cytoplasm coincides in time with the formation of collagen.

5. The scar tissue at the cut end of the nerve, which showed rapid formation of collagen comparable to that seen in healing skin wounds, revealed no significant alkaline phosphatase activity until after collagen had been extensively formed; the phosphatase was then associated with strands of Schwann cells that had wandered out from the end of the nerve.

I should like to express my thanks to Mr M. Abercrombie and Dr M. L. Johnson for suggesting this work and for their constant advice and encouragement during its progress. I am deeply grateful to Prof. G. R. de Beer for his supervision, and to Prof. J. Z. Young for helpful criticism of the work. I am also indebted to Mr F. J. Pittock for taking the photographs. The work was done while holding a research studentship from the Medical Research Council.

REFERENCES

Abercrombie, M. & Johnson, M. L. (1946*a*). Collagen content of rabbit sciatic nerve during Wallerian degeneration. *J. Neurol. Neurosurg. Psychiat.* **9**, 113–118.

Abercrombie, M. & Johnson, M. L. (1946*b*). Quantitative histology of Wallerian degeneration. I. Nuclear population in rabbit sciatic nerve. *J. Anat., Lond.*, **80**, 37–50.

Bodian, D. (1936). A new method for staining nerve fibres and nerve endings in mounted paraffin sections. *Anat. Rec.* **65**, 89–97.

Bourne, G. H. (1948). Alkaline phosphatase and vitamin-C deficiency in regeneration of skull bones. *J. Anat., Lond.*, **82**, 81–87.

Danielli, J. F. (1946). A critical study of techniques for determining the cytological position of alkaline phosphatase. *J. exp. Biol.* **22**, 110–117.

Danielli, J. F., Fell, H. B. & Kodicek, E. (1945). The enzymes of healing wounds. II. The effect of different degrees of vitamin-C deficiency on the phosphatase activity in experimental wounds of the guinea-pig. *Brit. J. exp. Path.* **26**, 367–376.

FELL, H. B. & DANIELLI, J. F. (1943). The enzymes of healing wounds. I. The distribution of alkaline phosphomonoesterase in experimental wounds and burns in the rat. *Brit. J. exp. Path.* **24**, 196–202.

FISHER, I. & GLICK, D. (1947). Histochemistry. XIX. Localization of alkaline phosphatase in normal and pathological human skin. *Proc. Soc. exp. Biol., N.Y.*, **66**, 14–18.

GOMÖRI, G. (1937). Silver impregnation of reticulum in paraffin sections. *Amer. J. Path.* **13**, 993–1001.

GOMÖRI, G. (1939). Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. exp. Biol., N.Y.*, **42**, 23–26.

HUNT, A. H. (1941). The role of vitamin-C in wound healing. *Brit. J. Surg.* **28**, 436–461.

LANDOW, H., KABAT, E. A. & NEWMAN, W. (1942). Distribution of alkaline phosphatase in normal and in neoplastic tissues of the nervous system. *Arch. Neurol. Psychiat., Chicago*, **48**, 518–530.

LISON, L. (1948). La recherche histochimique des phosphatases. Étude critique. *Bull. Histol. Tech. micr.* **25**, 23–41.

MASSART, L. & DUFAIT, R. (1942). Über die KCN und NaF-Hemmung der Gärung mit besonderer Berücksichtigung der Metalle als Aktivatoren der Fermente. *Hoppe-Seyl. Z.* **272**, 157–170.

MASSON, P. (1932). Experimental and spontaneous Schwannomas. *Amer. J. Path.* **8**, 367–416.

MELLORS, R. C. & SUGIURA, K. (1948). Alkaline phosphatase activity and basophilia in hepatic cells following administration of butter yellow to rats. *Proc. Soc. exp. Biol., N.Y.*, **67**, 242–246.

SHERLOCK, S. & WALSHE, V. (1947). Hepatic alkaline phosphatase: Histological and microchemical studies on liver tissue in normal subjects and in liver and in bone disease. *J. Path. Bact.* **59**, 615–630.

SULKIN, N. M. & GARDNER, J. H. (1948). The acid and alkaline phosphatase activity in the normal and recovering liver of the rat. *Anat. Rec.* **100**, 143–158.

TAKAMATSU, H. (1939). Histological and biochemical studies of phosphatase. *Trans. Jap. path. Soc.* **29**, 492–498.

EXPLANATION OF PLATES

With the exception of Pl. 2, fig. 5 and 6, which were frozen sections, the material was fixed in alcohol, embedded in paraffin and cut at a thickness of 7μ. Sections were then treated with Gomöri's histochemical technique for demonstration of alkaline phosphatase. Linear magnification is given in brackets.

Abbreviations: *m*, myelin debris; *n*, nerve stump; *s*, scar tissue; *S. c.*, Schwann cell cytoplasm.

PLATE 1

Fig. 1. Longitudinal section of undegenerated sciatic nerve (peroneal division) showing 'streaks'. Axons of large nerve fibres are also somewhat stained. (×105.)

Fig. 2. Transverse section of undegenerated sciatic nerve, showing 'streaks' cut across in interstices between large nerve fibres. (×615.)

Fig. 3. Longitudinal section of an undegenerated anterior mesenteric nerve, consisting mainly of unmyelinated fibres. Nuclei are stained, and also some fine fibrils which may be axons. (×680.)

Fig. 4. Section through an undegenerated anterior mesenteric ganglion. The ganglion cells show almost unstained cytoplasm, but the nuclei are darker with intensely stained nucleoli. Nuclei and fibres surrounding the ganglion cells are very intensely stained. (×645.)

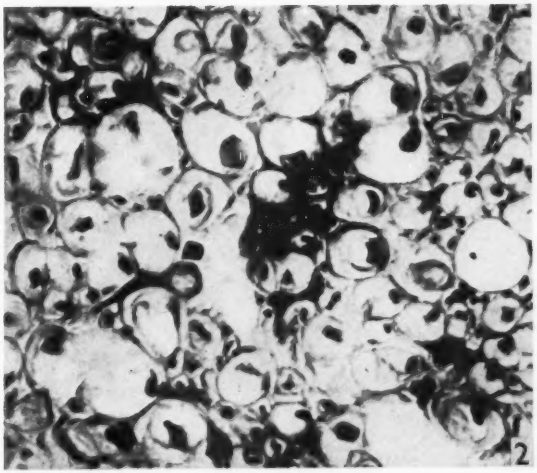
PLATE 2

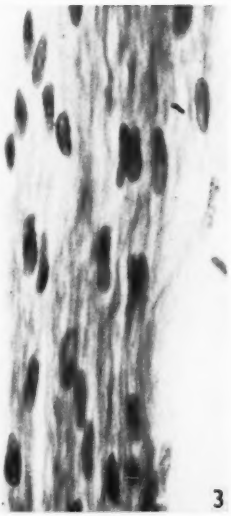
Fig. 5. Part of a frozen longitudinal section of a 2-day degenerated sciatic nerve in the peripheral region showing 'streaks'. Nuclei in this neighbourhood are somewhat stained. Axons and myelin framework of myelinated fibres are slightly stained. (×330.)

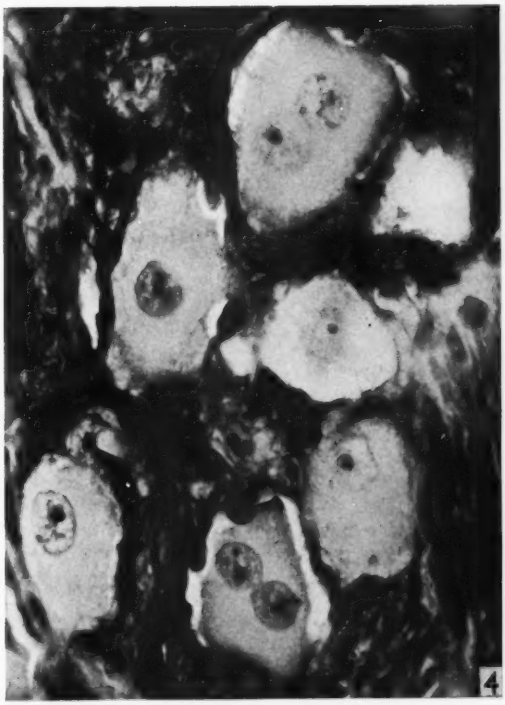
Fig. 6. Frozen longitudinal section through the traumatized region of a 2-day degenerated peroneal branch of the sciatic nerve. A cap of intensely staining polymorphs (*p*) lies over the cut end. (×20.)

Fig. 7. Longitudinal section of a 10-day degenerated sciatic nerve, peripheral region. Nuclear staining is more widespread than previously, and fibrous strands believed to be Schwann cytoplasm are also stained. Myelin debris (*m*) is still stained, but has become somewhat disorganized. Axons are no longer distinguishable. (×355.)

Fig. 8. Longitudinal section through the traumatized region of a 10-day degenerated sciatic nerve. The polymorphs have disappeared and the scar region (*s*) shows hardly any alkaline phosphatase activity, though collagen is rapidly being formed at this time. (×30.)



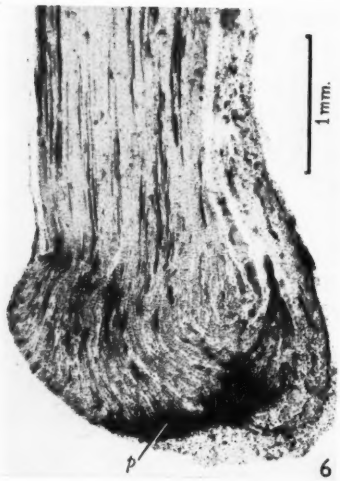




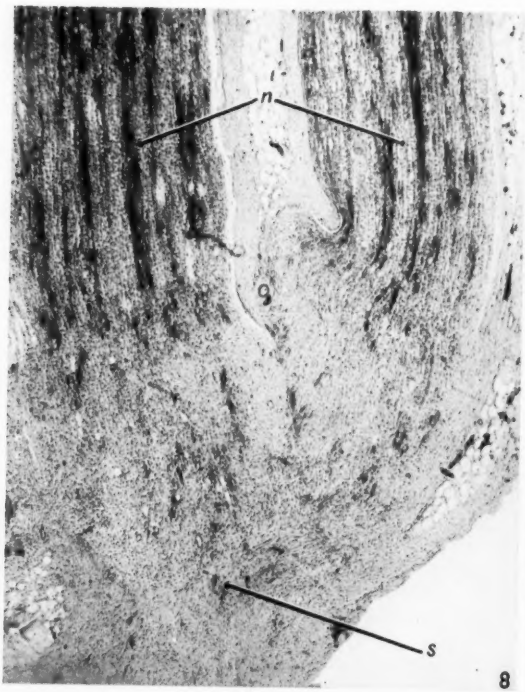
MARCHANT—ALKALINE PHOSPHATASE ACTIVITY IN THE NERVES OF THE RABBIT

5

7



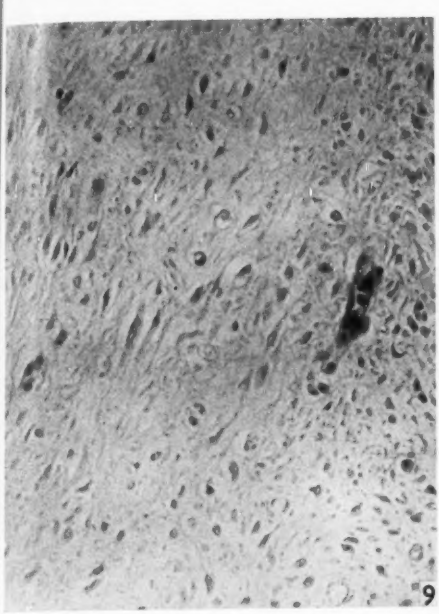
6

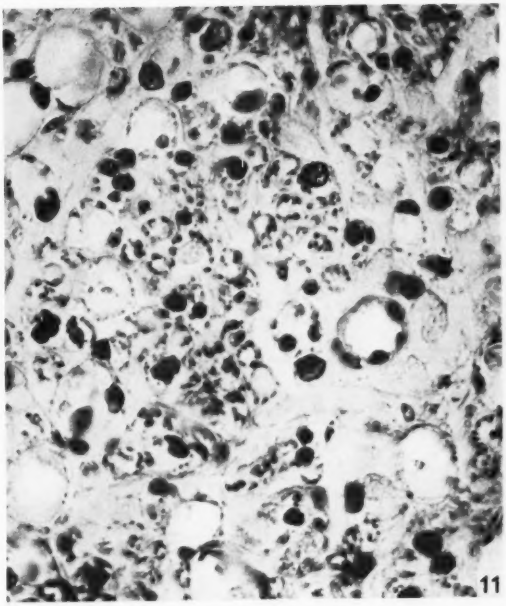


8

MARCHANT—ALKALINE PHOSPHATASE ACTIVITY IN THE NERVES OF THE RABBIT

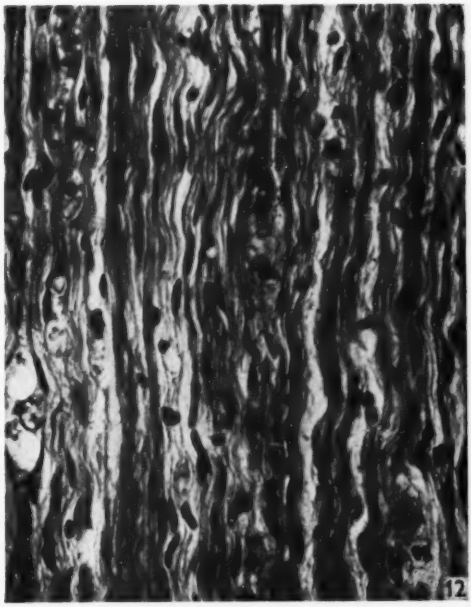
7

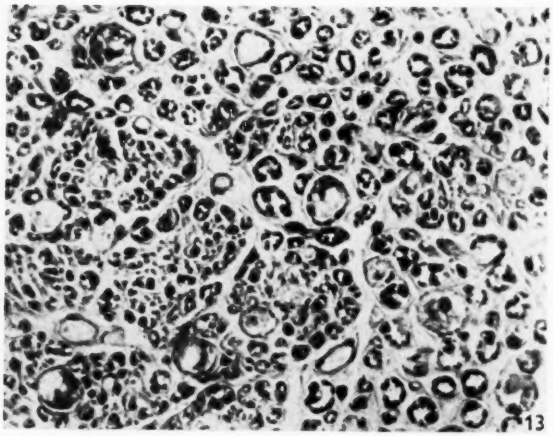

9


11

8

S.c.
10


12


13

n
s
14

15

PLATE 3

Fig. 9. Longitudinal section of scar tissue at the cut end of a 10-day degenerated sciatic nerve. Two leucocytes in a capillary are strongly stained. Otherwise the tissue is almost negative. (×405.)

Fig. 10. Longitudinal section of a 15-day degenerated sciatic nerve. Nuclei and fine longitudinal fibrils (probably mainly Schwann cell cytoplasm, *s.c.*) stained. Myelin debris no longer stained. (×455.)

Fig. 11. Transverse section of same nerve as in Fig. 10. Black dots on Schwann tube walls are probably Schwann cells. (×675.)

Fig. 12. Longitudinal section of a 100-day degenerated sciatic nerve. Fibrillar staining more strongly marked. (×445.)

PLATE 4

Fig. 13. Transverse section of same nerve as in Pl. 3, fig. 12. Black lumps within Schwann tube walls are probably Schwann cells. Endoneurial cells are also stained. (×425.)

Fig. 14. Longitudinal section through traumatized region of a 100-day degenerated sciatic nerve. There is intense staining of the Schwann cytoplasm, and the strands of Schwann cells (*s*), which have wandered out from the cut end of the nerve (*n*) can be distinguished from the surrounding connective tissue. (×18.)

Fig. 15. High magnification of scar tissue of the section shown in fig. 14. There is intense phosphatase activity in the strands of Schwann cells, but surrounding connective tissue is scarcely stained. (×290.)

OBSERVATIONS ON THE FEMALE REPRODUCTIVE ORGANS OF THE CA'AING WHALE *GLOBIOCEPHALA MELAENA* TRAILL

By R. J. HARRISON
Anatomy Department, Charing Cross Hospital Medical School

INTRODUCTION

The anatomy of the reproductive organs of the Mystacoceti has been described in detail by Mackintosh & Wheeler (1929), Ommanney (1932), and the literature has been reviewed by Mackintosh (1946). The cyclic changes occurring in the uterine mucosa of Balaenopterid whales have been the subject of a recent paper by Matthews (1948). His observations constitute the only detailed report, other than a brief reference by Mackintosh & Wheeler (1929), on the histology of the changes in the reproductive tract. Descriptions of the reproductive organs in the Delphinidae are, moreover, few in number, inconsistent, and give no reference to the histology of the ovaries or mucosa. Turner (1867) and Murie (1874) give a brief account of the immature vagina and uterus in *Globiocephala*. Meek (1918) describes the macroscopic appearances of the genital tract of *Phocaena communis*, now *P. phocaena* (four specimens), but there is little account of the ovary. The gross appearances of the genital organs of an immature *Delphinus delphis* are described by Pycraft (1932), and Khvatov (1938), quoted by Asdell (1946), who states that the corpora lutea are still present and show no sign of involution at the time of parturition. Comrie & Adam (1938) give an account of the female reproductive system of *Pseudorca crassidens*. They also describe the ovaries from fifteen females, giving particular attention to the corpora lutea, but making no histological observations.

The following observations on the female reproductive organs of *Globiocephala* are therefore presented to add to the scanty knowledge of a little described species. The histological appearances of the ovary, the corpus luteum and the mucosa of the uterus have not previously been described. Unfortunately, insufficient material does not permit of more than a partial comparison with the changes described by Matthews (1948) in Balaenopterid whales.

MATERIALS AND METHODS

The material to be described was obtained from a school of some 300 whales killed at Torshavn, in the Faroe Islands, in March 1947. Due to the kindness of Dr Carl Bech of Torshavn a representative selection of ovaries and genital tracts was removed from the females, and was at once fixed in 12% formalin.

The lengths of the majority of the whales from which the ovaries were taken are not known. However, Dr Bech states, in a personal communication, that the newborn young are 3–3½ ft. in length, that immature specimens are of the order of 9–11 ft. in length, and that adult females are up to 16 ft. in length. The material obtained consists of the infra-umbilical part of one 42 cm. foetus with the repro-

ductive system intact, the genital tracts of twelve females, mostly immature, and the ovaries of thirty-eight females. The size of the ovaries and three mean diameters of the corpora lutea and corpora albicantia are given in Table 1.

Representative portions were taken from the ovaries and, after sectioning, were variously stained with Harris's haematoxylin and eosin or van Gieson's stain, with Mallory's triple stain and by the methods described by Rossman (1942) for luteolipin. Portions of the uterine tube, uterine horns, fused horns, uterus and vagina were also sectioned and stained. It was found that immersion, prior to embedding, in 4% aqueous solution of phenol, with the addition of a little glycerine, facilitated the sectioning of the hard fibrous tissue without distorting the appearances.

THE REPRODUCTIVE ORGANS

The appearances of the external genitalia do not differ in any essential from the description given by Murie (1874):

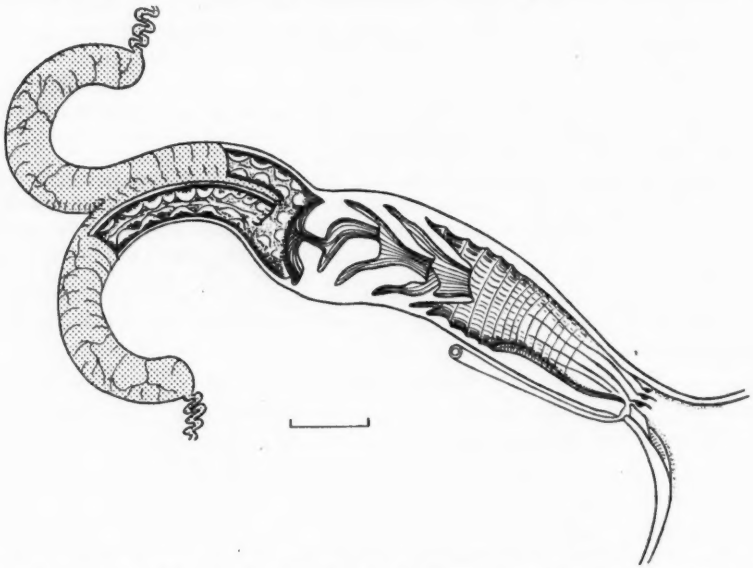
> The female external generative aperture is an elliptical fissure...near the middle of the third fourth of the animal's length, and inferiorly about vertical to the termination of the panniculus. The tegument around is dark coloured, and thrown into a good number of minute parallel and wavy wrinklings chiefly transverse in direction....Immediately within each tip of the vulva, or what may be considered the labia majora, are some twenty or more short, but deep, folds of membrane, in the recesses of which are crypts, the openings of sebaceous glands. The homologues of the nymphae or labia minora are two prominent folds of the mucous membrane, each one inch in length, which lie within the anterior pudendal commissure, and slightly converge as they pass backwards. Between these and with lateral plicate sulci is a median ridge 1·3 inches long, which ends in a small but distinctly pronounced clitoris.

In the 42 cm. foetus (Text-fig. 1) the wrinkling of the skin about the vulva is not present, and there are only six folds within the tip of the vulva, and their position is mainly lateral. The opening of the vulva is 2·0 cm. in length and 0·5 cm. in width; the junction of the vulva and vagina is markedly constricted. The vagina is 6·0 cm. in length, and it widens to 2·0 cm. in diameter at its upper portion, but narrows again at its upper limit. Immediately anterior to the opening of the urethra at the base of the clitoris lies a well-marked slightly raised area, in the form of a pear-shaped plaque 2 × 1 cm. on the ventral wall of the vagina. The surface of this plaque is only slightly grooved by shallow longitudinal fissures. In the maturing specimen the area is no longer raised in the form of a plaque and the vaginal wall in this lower portion is similar in its appearance to that of *Balaenoptera physalus* (Ommanney, 1932).

The structure of the upper part of the vagina is described by Murie as follows:

> The upper half, on the contrary is narrower and has a very uneven surface. This roughening depends on a numerous series of transverse rugae or puckerings of the membranes, some four of which are extremely prominent. In alluding to these valvular folds, Hunter (1787) aptly compares them to a succession of ores tincarum. They are composed of thick induplications of the fibroid tissue of the wall of the vagina inwardly lined by narrow longitudinal mucous rugae, which fringe their free edges. The fold nearest to the os, and only a thumb's breadth from it, has a thickness of 4/10 of an inch. The true os uteri is only distinguishable from the preceding folds by its narrower and somewhat firmer ring-like aperture.

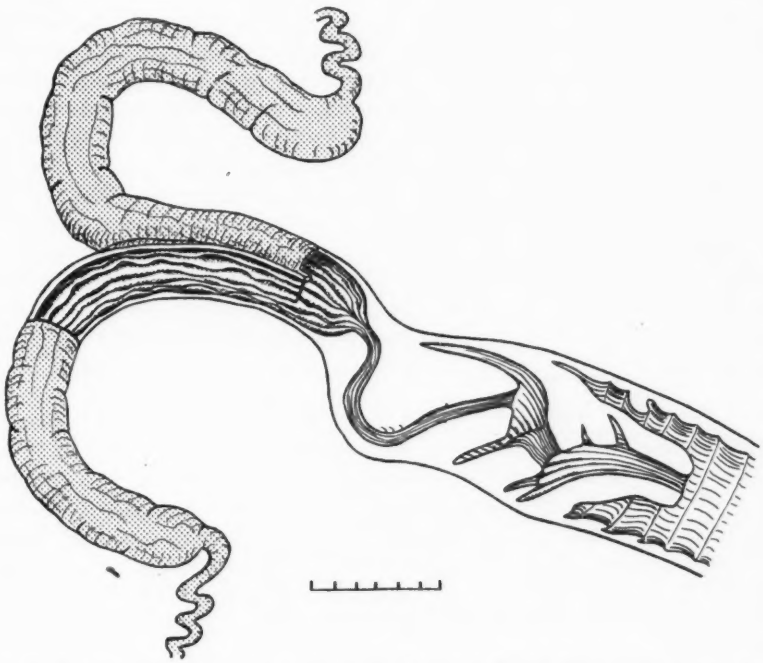
The appearances of the vagina in the 42 cm. foetus and in the adult are shown in Text-figs. 1 and 2. It will be seen that the uterine cavity opens through a small aperture into a narrow canal, each lined with mucous membrane that is thrown into numerous longitudinal folds. The canal has a curved course for about 8 cm. in the adult and ends by opening through a large conical projection. It is considered that this canal represents the true cervical canal and that the conical projection is the cervix. Below the cervix there is a series of large transverse folds, two of which entirely encircle the vagina, and thus create two chambers. The cervix projects into the upper chamber, which leads into the lower through a narrow aperture. The lower chamber communicates with the lower part of the vagina through a further narrow opening in the second vaginal fold. The lower vaginal folds are drawn down



Text-fig. 1. Drawing of the reproductive tract of the 42 cm. foetus. The vagina and cervix have been sagittally sectioned and parts of the posterior walls of the fused horns and left horn have been removed. The scale represents 1 cm.

into the vagina, so that they are similar in appearance to the cervix. The two main vaginal folds have secondary leaf-like folds arising from them. One such secondary fold is present in the 42 cm. foetus (Text-fig. 1), and a varying number up to four are found in the immature and mature specimens (Text-fig. 2). All the folds are lined on their inner aspect by a series of parallel longitudinal rugae. Microscopical examination of the vagina and its folds, both in the foetus and in the adult shows them to be very muscular structures. A thin outer longitudinal muscle layer is present, which sends fasciculi up into the bases of the vaginal folds. The deep muscle layer is circular, well developed and arranged in a series of bundles separated by fibrous tissue. In the vaginal wall the bundles are small and numerous, but in the vaginal folds and cervix they are large and separated by much fibrous tissue.

The uterus is relatively small, being 8 mm. in length in the 42 cm. foetus and 15 mm. in the newly born specimens. The walls of the uterine cavity are smooth, with a few raised protuberances or cotyledons. In the immature specimens the chamber measures 5·0 cm. in length and in the adult it is 8 cm. in length by 5·5 cm. in breadth. In Murie's specimen, an immature female, the uterus measured 5 cm. in length. The mucous membrane in the adult is raised into numerous longitudinal folds or rugae. These rugae are continuous with similar ones in the uterine horns and they narrow at the internal os and are continuous with the folds lining the cervical canal.



Text-fig. 2. Drawing of the upper reproductive tract of the whale CW 11. The tract has been sagittally sectioned up to the level of the fused horns; one side of the fused horns and part of the left horn have been opened by removal of the posterior wall. The scale is in centimetres.

The uterine horns are fused for the lower 20 mm. in the newly born and for the lower 10 cm. of their extent in the adult. The free part of the uterine horn measures 9·0 cm. in the newly born and 25 cm. in the adult. The mucous membrane of the foetal horn is raised into a series of cotyledons and elongated rugae. However, in the adult the mucous membrane is raised into a number of parallel longitudinal folds about 2 mm. in width and 4 mm. in depth. In places these folds are interrupted, and it is apparent from the intermediate stages that the small cotyledons of the foetal and immature specimens become drawn out into the long parallel folds of the adult (Text-figs. 1 and 2). The broad ligament is attached to the postero-lateral

aspect of the uterus and fused horns, the main bulk of the ligament passing behind the uterus. However, the ligament is attached to the lateral border of the separate uterine horns, and the first part of the uterine tube pursues its sinuous course in the substance of the broad ligament. Daudt (1898) describes a strong band running from the rostral end of the ovary to the foremost end of the ostium abdominale; Ommanney (1932) describes the same band in *B. physalus*. The band is also present in *Globiocephala*, as also is a strong band running from the posterior aspect of the ovary to the uterine horn, considered by Daudt to be the ligamentum ovarii. The ligamentum teres is also present as a strong band running in the broad ligament from the base of the uterine horns to the inguinal canal.

In the material under discussion the uterine tube can be divided into three parts. The first part in the adult is sinuous and thick walled, and when drawn out extends for 6–10 cm. This leads into a wider, straighter, thin-walled part, which runs along the supero-medial aspect of the ovary to its anterior end. Here the tube opens out by a funnel-shaped pouch which overhangs the ovary (compare with the findings of Pycraft (1932) in the dolphin). The free lower and medial edge lies below the ovary so that the latter is virtually enclosed in a pouch, reminiscent of the ovarian sac of the seal. The inner aspects of the pouch and infundibulum are lined by numerous membranous ridges, which run downwards towards the ostium. These are presumably the fimbriae mentioned by Murie (1874). Microscopical examination shows that the inner aspect of the pouch is lined with small villous projections, covered with ciliated columnar epithelium and containing a narrow central core of delicate connective tissue.

Histological changes in the reproductive tract

Unfortunately there is insufficient material for an adequate report to be made on the changes occurring in the reproductive tract during the cycle, or during pregnancy. However, the complete tracts of two whales, CW 10 and CW 11, are available for examination. The first, CW 10, contained no corpora lutea in either ovary, and the presence of only a few small follicles less than 1 mm. in diameter indicates that it is an adolescent specimen. The whale CW 11 contained the earliest corpus luteum of the series in one ovary; both ovaries displayed marked follicular development.

The mucosa, as Matthews (1948) describes in the Balaenopterid whales, is clearly divided into two layers. The outer subepithelial layer is composed of a thin stratum compactum, varying from 150 μ thick in CW 10 to 400 μ thick in CW 11, which contains the ducts of the glands and a superficial capillary plexus together with a few larger vessels. The deeper layer, the stratum spongiosum, is thicker, comprises the rest of the mucosa and contains the convoluted glands. The cells of the stratum compactum are relatively small, and tend to be stratified, giving the layer a distinctly more dense appearance than that of the stratum spongiosum. The cells of the latter layer are larger and are very loosely arranged. The epithelium has been retained over most of the mucosa, but in many areas, particularly of the mucosa of the body of the uterus, the epithelium has been lost. The writer agrees with Matthews (1948) that this loss of the epithelium is probably a post-mortem change occurring before fixation.

The uterus of the adolescent specimen CW 10 (Pl. 1, figs. 1 and 2) has its lining raised into a series of rugae 1–2 mm. high and 1 mm. thick; similar, but larger, elongated rugae are found in the fused horns and in the horns. The epithelium is low cuboidal or cubical, the nuclei are darkly stained and occupy most of the cell. The ducts are lined by high cubical or columnar epithelium and there is no visible lumen. The glands are straight with darkly staining nuclei and little cytoplasm; the lumen is small or not visible. The stroma is dense and compact, all the cells stain heavily, there is no distinction of the stroma into two layers. The blood vessels are small and straight and there is a number of small vessels anastomosing just beneath the epithelium.

In CW 11 (Pl. 1, figs. 3–5) the lining of the uterus, fused horns and horns shows distinct changes. The epithelium is of the high columnar pseudo-stratified type and the cytoplasm is increased in amount. The endometrium of the uterus is now 3–4 mm. thick and the rugae are correspondingly enlarged in the horns. There is a distinct stratum compactum, containing the ducts of the glands, and numerous dilated anastomosing blood vessels. The stratum spongiosum is oedematous and the stromal cells are enlarged. The glands are hypertrophied, distended and coiled, and the basal part shows evidence of active proliferation. The lumen of each duct is dilated and active secretion is commencing in the glandular epithelium. The arteries are enlarged and tortuous, and the superficial vessels are engorged. In the superficial part of the stratum spongiosum the glands are larger in diameter, and the lumina are also larger. The glands in the mucosa of the uterus and the fused horns are larger in diameter than those of the horn. Small depressions can be seen in the surface of the mucosa, but because of the lack of material from later in pregnancy these cannot definitely be called incipient crypts, such as Matthews (1948) describes in Blue and Fin whales.

Transverse sections of the uterine tubes (Pl. 2) show that the epithelium is of the pseudo-stratified columnar type. The epithelium of the tube of CW 10 is low cuboidal or columnar and the lumen is open and regularly stellate. In CW 11 the epithelium has changed to the high columnar type, the lumen is irregular and the epithelium more plicated. The epithelial cells are hypertrophied and the submucosa is oedematous.

It is therefore evident that the corpus luteum of CW 11, despite its small size and relatively early histological development, has initiated progestational changes in the reproductive tract.

THE OVARY

The ovary is suspended by a short thick mesovarium on the inner side of the broad ligament. In the foetus and the adolescent specimens the ovaries are white, elongated, oval bodies with smooth surfaces. There are no 'bramble' markings present, such as are found on the ovary of *Balaenoptera* (Ommanney, 1932). In the adult specimens containing a fully developed corpus luteum the shape of the ovary is almost spherical, due to the relatively greater mass of luteal tissue. The colour of the adult ovaries becomes a dark grey as the animal ages. The ovary is divided into cortex and medulla, the latter consisting of tortuous, interlacing arteries and veins embedded in a dense mass of white fibrous tissue. The ovarian artery divides before

reaching the hilum into a large number of vessels, each accompanied by one or more veins. The main blood supply passes into the medulla, but there are several small branches passing into the cortex. The cortex is relatively broad in extent and is filled with developing and atretic follicles embedded in the stroma. The tunica albuginea is thick, but it does not prevent protrusion of maturing follicles, or considerable herniation of the young corpus luteum. No interstitial cells have been observed in the medulla or the cortex.

In the hilum and proximal part of the medulla of the foetal ovary cords of poorly differentiated mesenchymatous cells are present. They probably represent the remnants of the rete ovarii. Remnants are also found in the ovaries of adolescent specimens. The cortex of the foetal ovary contains numerous primary oocytes, each oocyte being enclosed by a single layer of deeply staining flattened granulosa cells. The diameter of these primary oocytes is from 25 to 35 μ.

Large numbers of primary oocytes are found in the ovaries of the newborn and recently born specimens. Many of these oocytes show signs of impending or advanced degeneration. The ooplasm is distorted and vacuolated, and in many instances the oocyte has completely disappeared, leaving a flattened cavity surrounded by degenerating epithelial cells. There is no evidence of follicular growth in the youngest immature specimens.

The ovaries of the youngest specimens can be divided into three main groups from consideration of their size and volume, histological appearance and the size of the parts of the reproductive tract. The first group contains the ovaries of the newly born specimens (3) (ovary length 2·1–2·4 cm.), the second consists of the ovaries of the immature specimens (4) (ovary length 2·6–3·2 cm.), the third comprises the ovaries from adolescent specimens (7) (ovary length 3·8–4·5 cm.). None of these ovaries contains a corpus luteum and only the last group contains developing follicles.

The ovaries from the third group of adolescent specimens (ovary length 3·8–4·5 cm.), all show marked follicular development. Large numbers of small and medium-sized follicles are present and have a diameter varying from 0·5 to 6 mm. In two of the larger ovaries of this group large follicles are present, with a diameter of 2–2·5 cm. The largest follicle (fixed) seen in the whole series had a diameter of 3·25 cm. and was in the ovary that contained the youngest corpus luteum (CW 11). The size of this follicle, when compared with that of the recently formed corpus luteum, indicates that the size of the mature follicle prior to ovulation is probably in the region of 3–4 cm.

Microscopical examination of the ovaries of this third group shows the presence of small numbers of healthy primary oocytes, 25 μ in diameter, in the cortex; there are also numbers of degenerating oocytes. No polynuclear oocytes or multiovular follicles have been seen in any of the ovaries of the series. The structure of the maturing follicle is of the typical mammalian form, and there is marked development of the theca interna in the medium-sized follicles (Pl. 3, fig. 2). In many places developing follicles are surrounded by a theca interna layer some twelve cells thick. These cells show all the changes associated with glandular activity. The nuclei are enlarged and show vesicular changes, and vacuolation of the cytoplasm is a marked feature. Many developing follicles possess thecal cones (see Strassmann,

1941; Harrison, 1948) on the pole of the follicle nearest the ovarian surface. Some of the smaller follicles (0·3–1 mm. in diameter) have internal cones on the pole of the follicle nearest the medulla of the ovary. The possible significance of the transient appearance of these inwardly directed cones of thecal cells has been discussed in a previous paper (Harrison, 1948).

Although there is insufficient material for a statistical analysis it appears that the growth of the ovum and follicle follows the usual two well-marked phases described (Parkes, 1931). The antrum develops when the follicle is 250–350 μ in diameter, and the zona pellucida appears at about the same stage, the oocyte being then of the order of 70 μ in diameter.

The granulosa cells in the healthy follicle show a basal layer of columnar cells, their nuclei arranged regularly with the long axis of each nucleus perpendicular to the lamina propria. The inner layers of the granulosa cells are cubical or spherical in shape and the nuclei are irregularly placed. Small spaces resembling the bodies of Call and Exner can be seen in the granulosa layer of maturing follicles (Pl. 3, fig. 2). Many of the follicles in the adolescent ovaries show signs of atresia. The even columnar arrangement of the basal layer of granulosa cells is disturbed, and this change coincides with the first appearance of degenerative changes in the oocyte. The granulosa cells lose their compact arrangement and the layer becomes loosened, the cells swell and subsequently degenerate. A further characteristic sign of early degeneration is a change in shape of the follicle. The peripheral pole of the follicle shows a tendency to collapse inwards, giving the follicle a pear-shaped appearance. The granulosa cells in the collapsed part of the follicle tend to remain undegenerated longer than those at the uncollapsed medullary pole. This collapse of the outer pole of the follicle has not been seen so markedly in ovaries of any other mammal examined, and it is apparently mainly limited to the ovaries of adolescent specimens. It is possible that it is due to the fact that the fibrous tissue of the medulla gives some stability to the inner pole of the follicle, whereas the actively growing stroma in the cortex, together with the presence of numerous follicles at various stages of development, exert a local pressure on the outer pole.

THE CORPUS LUTEUM

In the available material there are five ovaries which contain recently formed or fully developed corpora lutea (Pl. 3, fig. 1). Three of these corpora are from whales which had recently ovulated. Careful examination of the reproductive tract of the earliest of these specimens failed to reveal any products of conception. The details of the ovaries containing these corpora are given in Table 1.

The smallest of the corpora lutea (CW 11) has a diameter much less than that of a mature follicle, and there are no corpora lutea in the other ovary. There is considerable herniation of the corpus luteum, the herniated part being a nipple-like projection with a slit, 4 mm. long at the apex of the projection. This slit, which is slightly depressed into the substance of the corpus luteum, marks the stigma. The cut surface of the corpus luteum is a pale yellowish grey in colour. The collapsed and folded mural epithelium is clearly seen; there is no marked haemorrhagic effusion into the centre (Pl. 3, fig. 1).

Microscopical examination shows a markedly folded epithelium. Considerable

trabeculation is present; projections of theca externa containing thecal vessels form the central core of the trabeculae (Pl. 3, fig. 3). A small slit-like central cavity is present, lined by fibroblasts. Large groups of theca interna cells are to be seen at the bases of the trabeculae and at the periphery of the developing gland. Some thecal cells have been carried into the developing gland about the trabeculae. The theca interna cells are relatively small, with a slight amount of cytoplasm containing several minute vacuoles; the nuclei are densely stained. The cells are smaller than

Table 1. *Measurements of ovaries and corpora lutea in adult specimens*

Animal no.	Ovary Length (mm.)	Breadth (mm.)	Depth (mm.)	Volume (c.c.)	Corpora lutea* Mean of three diameters (mm.)	Follicles
CW 11	50	30	25	15·5		
	55	30	20	16·0	18	Many small, several 1–3 cm.
4	42	34	32	26·0	33	Many small, several 1 cm.
	40	22	24	21·0		
16	80	25	15	24·0	20	None larger than 1 mm.
12	65	22	23	16·0	18	Many small, several 1 cm.
27	65	30	25	27·0	13	Many small, few 5 mm.
	64	30	26	28·0	18	
19	55	25	24	20·0	12	Few less than 1 mm.
21	50	30	20	13·0	7	Few less than 1 mm.
1	60	60	40	88·0	50, 23	None
	55	30	28	22·0	17	
30	90	30	15	29·0	18, 17, 17	Few very small
25	65	25	25	24·0	16, 13, 9	Few very small
28	55	35	20	27·0	15, 9, 9, (? 1)	Many 5–10 mm.
15	64	35	24	35·0	14, 12, 11, (? 2)	Few 5 mm.
22	45	30	20	13·0	13, 12, 9, (? 2)	Few very small
	50	25	22	12·5	12, 8	
24	70	30	25	30·0	7, 5, 3, (? 3)	Few very small
3	60	40	20	32·0	20, 15, 14, 12	None
	55	35	20	32·0	17, 13	
23	55	30	20	18·0	13, 12, 10, 7	Two 3 mm., few very small
26	65	30	35	30·0	17, 14, 10, 7, 6	Few small
20	65	35	30	24·0	15, 12, 9, 8, 5, (? 1)	Few small, one 10 mm.
18	55	25	18	16·0	12, 9, 7, 5, 4, (? 1)	Few small
2	70	50	30	64·0	30, 21, 18, 13, 10, (? 1)	None
5	70	60	35	66·0	41, 17, 11, 9, 7, (? 1)	None
	65	30	25	21·0	13, 12, 7, (? 2)	
29	50	30	18	24·0	13, 8, 8, 8†, 7, 6, (? 1)	Few very small

* The figures in brackets indicate the probable number of further corpora lutea, which are not visible to the naked eye, but can be seen in histological sections.

† See p. 248.

those found at the periphery of developing follicles. The granulosa cells show varying degrees of lutealization. Those cells near the periphery of the gland have an average diameter of 15 μ compared with that of 10 μ for the follicular granulosa cells. The majority of the cells have a granular eosinophilic cytoplasm, some cells already showing evidence of vacuolation. The vacuoles are either numerous, small and spherical, or single, large and oval. The nuclei of the granulosa cells are for the most part small and darkly stained, a few show vesicular changes. The granulosa cells are loosely arranged, being separated by fluid-filled spaces. There is some

fibrin between the cells and numbers of erythrocytes are also present in the fluid-filled spaces. Vascularization of the gland has commenced and small sinusoids and capillaries have been formed from the thecal vessels. Endothelial cells can be recognized invading the peripheral part of the gland. Early reticulum deposition has commenced in the peripheral part of the gland.

The general appearance of this early corpus luteum is therefore of an open 'lace-like' arrangement of lutealizing granulosa cells, such as has been described by Pearson & Enders (1943) in the fox, and by Harrison (1946) in the mare. It is apparent that ovulation has only recently occurred, approximately within the previous 48 hr., and from comparison with the appearances with those of other mammals, probably within 24 hr. The size of this corpus luteum, with a mean diameter of 19 mm., suggests that the size of the original mature follicle was of the order of 3–4 cm. in diameter. Comparing this tentative size of the mature follicle with that of other follicles present in the ovary, it appears that in *Globiocephala* only one or two follicles enlarge to a maximum diameter prior to ovulation.

The second and third specimens (CW 2 and CW 4, Table 1) are similar in appearance and structure, and the corpora lutea are approximately at the same stage of development. Both corpora lutea are markedly herniated, and the stigma is more depressed than in the previous specimen. A vertical slit passing through the centre of the gland, marks the remnants of the central cavity. The cut surface is yellower than the previous specimen, but the lobulated structure is still apparent (Pl. 3, fig. 1).

Microscopically the trabeculae have increased in thickness, mainly due to invasion by theca externa cells and increase in size of the vessels. The theca interna cells are still present as groups of small eosinophil cells, their cytoplasm devoid of vacuoles and their nuclei darkly stained. These groups are mainly arranged in the vicinity of the trabeculae, but some cells have wandered out between the luteal cells.

The granulosa cells have changed considerably and have increased in size to an average diameter of 30 μ. The cytoplasm has increased greatly in amount and also in granularity. Some cells have an evenly granular cytoplasm, but others show varying degrees of vacuolation. In some cells the peripheral cytoplasm is full of small vacuoles, giving it a honey-comb appearance. The peri-nuclear cytoplasm is dense and granular. A striking characteristic of many luteal cells is the presence of two or more nuclei in one cell. These multinucleated cells are large, having a diameter of over 40 μ (Pl. 4, fig. 1). Their nuclei are large and vesicular, but many of them are darkly staining, with the chromatin gathered into coarse granules. Occasional mitotic figures can be observed. There is little doubt that at this stage in the development of the corpus luteum there is mitotic division of the nuclei of the luteal cells. The cytoplasm of the cells does not necessarily divide, however, and, as a result, large multinucleated luteal cells are formed. The luteal cells tend to be arranged in columns, some four or more cells in breadth, in which many of the cells exhibit this multinucleated condition. The luteal columns are loosely arranged and are separated by fluid-filled spaces (Pl. 4, fig. 2). Endothelial cells are to be seen invading these spaces and vascularization of the corpus luteum is taking place. In many places, near the periphery, endothelial sinusoids have been formed around groups of luteal cells.

The fourth corpus luteum (CW 1) to be described is larger than the previous specimens and the cut surface has a pale ochre colour (Pl. 3, fig. 1). The gland is pear-shaped, markedly herniated, and a broad dimple marks the position of the stigma. The trabeculae have further increased in size, giving the gland a lobulated appearance on section. This corpus luteum is one of pregnancy, but the foetus was, unfortunately, not accurately measured in the field.

Marked histological changes have occurred. The trabeculae are thicker and consist of fibrous tissue, and the blood vessels in them have thick walls (Pl. 4, fig. 3). The luteal cells are collected into columns or groups by a network of fibrous tissue. The vessels running in the fibrous network are flattened and contain little blood. The luteal cells are smaller, having an average diameter of 25 μ. Multinucleated cells are still to be seen, but all the nuclei are smaller and darker staining than in the previous specimens. Very few of the cells are vacuolated, and the cytoplasm is evenly granular in all the cells. At the periphery of the gland small irregularly shaped cells with pale eosinophil cytoplasm and small darkly staining nuclei can be seen interspersed amongst the luteal cells. It is presumed that these cells are the theca interna cells. Such a statement cannot be verified without following the changes through a number of stages, but it is unlikely that such a series will be obtained.

The last specimen (CW 5) is slightly smaller than the previous one and shows signs of impending retrogressive changes (Pl. 3, fig. 1). The specimen was obtained from a pregnant animal, but the length of the foetus is not known. The fibrous matrix is even more pronounced and there is evidence of shrinkage of the luteal cells (Pl. 4, fig. 4). None of the luteal cells contain vacuoles and the cytoplasm has a 'fibrous' appearance. Many of the nuclei are pycnotic or fragmenting. There is little blood in the vessels of the gland, and the majority of the large vessels are developing thick walls. Multinucleated cells are present, but are infrequent. Theca interna cells can still be seen at the periphery of the gland and their appearances are the same as in the previous specimen.

This corpus luteum is considered older than the previous specimen on account of the shrinkage of the luteal cells, and the signs of retrogressive changes.

In the ovary of CW 29 a small yellow body 10 mm. long, 8 mm. wide and 5 mm. in depth was seen. Unfortunately the other ovary was not obtained. This body was sectioned and was found to have a structure resembling an active corpus luteum. The histological appearance is of a corpus luteum older than that of CW 4 and younger than that of CW 1. There is no trabeculation and little fibrous tissue in the gland. Typical luteal cells are present, some containing small spherical vacuoles in the peripheral cytoplasm. Multinucleated luteal cells can be seen and the nuclei are all vesicular. Theca interna cells with their typical appearance are present at the periphery of the gland. The structure is very vascular and the vessels are distended with blood. The appearances thus indicate an apparently active area of luteal tissue. It is noteworthy that in the same ovary there is a larger, though degenerated, corpus luteum of some previous cycle or pregnancy. The ovary contains no follicles. The lack of knowledge of the state of affairs in the other ovary prevents anything but a tentative suggestion as to the nature of this structure. However, it is possible that it is an accessory corpus luteum, derived by the lutealization of a small follicle.

THE RETROGRESSING CORPUS LUTEUM

It is well known that in the Mystacoceti the corpus luteum of each ovulation persists in a degenerated form for a long time and possibly throughout the life of the whale (Mackintosh & Wheeler, 1929; Mackintosh, 1946, for review). Comrie & Adam (1938) have investigated the retrogression of the corpora lutea of *Pseudorca crassidens*, and their results will be discussed in association with the findings in *Globiocephala*.

In the material available eight ovaries contain one retrogressing corpus luteum, two contain two, seven contain three, four contain four, and four contain five, in addition to the five recently formed or fully developed corpora lutea already referred to. All the retrogressing corpora lutea are of different sizes; but their histological appearances are the same. Externally they are apparent as raised, puckered plaques on the surface of the ovary with a wrinkled ridge or ridges around the raised button-like area. The largest old corpus luteum has a mean diameter of 23 mm. and the smallest discernible to the naked eye is 3 mm. in mean diameter. Below this size old corpora lutea raise no wrinkles on the ovarian surface. Sections of the cortex of the ovaries containing many old corpora show the presence of further areas simulating the appearance of a degenerating corpus albicans. Therefore, although remnants of corpora albicantia remain visible for a long time in *Globiocephala*, it appears that such remnants shrink to a degree which does not enable them to be seen on the surface of the ovary. It is also to be noted that one of the ovaries (CW 24) which, by its dark colour and atrophic appearance, is from an old individual, contains visible remnants of only three old corpora lutea. These remnants are 7, 5 and 3 mm. in mean diameter and have thus undergone retrogressive changes for a considerable period. However, microscopical examination of areas of the cortex revealed at least three further old corpora lutea of a mean diameter of 3, 2 and 2·5 mm., and which were invisible to the naked eye. It thus appears that retrogression in the corpus luteum and in the corpora albicantia is essentially similar to that occurring in other mammals, except that the retrogressive process extends over a greater length of time. The corpora albicantia eventually shrink to a size unappreciable by the naked eye, and they may presumably disappear entirely.

In one of the specimens (CW 30) three of the corpora albicantia are of approximately the same size and are arranged in a row, each being a pedunculated mass on a broad stalk.

DISCUSSION

An arrangement of folds in the upper part of the vagina resembling the condition in *Globiocephala* has been described by Pycraft (1932) in other species of Delphinidae, and this author gives the term 'pseudo-cervix' to the elongated and conical appearance of the vaginal folds. He comments on the wide range of variation in the details of the anatomy of the uterus and vagina in the Delphinidae. Ommanney (1932) gives a selection from the accounts of various authors showing the variation in the number of the vaginal folds. They vary in number, not only in the species, but also in the individuals of a single species. Daudt (1898) describes nine to twelve folds in *Phocaena phocaena*, whereas Meek (1918) finds only two great folds. Daudt (1898) finds twelve folds in *Balaenoptera physalus*, whereas Ommanney (1932) finds six folds. The appearances in *Globiocephala* suggest that there is a primary pattern of

two entire folds below the cervix, to which secondary folds are added, possibly during the growth of the whale.

Murie (1874) describes four vaginal folds in *Globiocephala*, but one of these folds is apparently the external os of the cervix opened up. His illustration, in fact, shows the internal os, the cervical canal and the external os, and two complete vaginal folds. This interpretation shows an arrangement of the vaginal folds similar to that found in the specimens under discussion. Turner (1867) remarks, indeed, that the lips of the cervix are liable to be confounded with the upper transverse folds of the vagina.

Meek's suggestions (1918) that the penis probably penetrates the vaginal folds and that their function is to promote the emission of seminal fluid appear to be correct. It is also probable that they assist in conveying the seminal fluid towards the uterus by their muscular contractions. The seminal fluid is able to collect in the chambers formed by the vaginal folds, and their arrangement, and also the relative narrowness of the vulva will prevent sea water entering the uterus.

The killing of so many members of one school of *Globiocephala* at the same time enables some information to be obtained on the reproductive pattern. There are present in this one school on the same day at least one animal that has recently ovulated, two which have ovulated some days previously, two which are approximately in the middle of the gestation period and one full-term foetus (not obtained for this report). There are also seven recently born or immature females and nine adolescent females, the ovaries of the latter showing varying degrees of follicular development. The remaining ovaries contain several corpora albicantia visible to the naked eye and varying in number from one to five. Remnants of further corpora albicantia can be found on microscopical examination. It thus appears that there is no definite breeding season, although it is possible that ovulation does not occur during the winter months. However, so small a series does not completely rule out such a possibility. If it is assumed that the gestation period is from 9 months to a year (the gestation period of the Common Ocean Dolphin is said to be 276 days by Khvatov (1938) quoted by Asdell (1946)), the pregnant whales ovulated during the summer months of 1946, and the first group of newly born females was born in the early spring of 1947, and the adolescent females during the previous year. This estimate would suggest that the whale CW 11 was born in 1945 and has thus reached puberty at the end of its second year.

It is agreed with Comrie & Adam (1938) that the site of ovulation appears to be situated anywhere on the surface of the ovary. There is no tendency for the mature follicle to rupture at the poles of the ovary as in the mare (Harrison, 1946). Comrie & Adam note a greater retrogressive effect on the corpora albicantia in ovaries containing a corpus luteum of pregnancy. In the specimens of *Globiocephala* no such increase in the retrogressive process is noted. Corpora albicantia in the ovaries of CW 1 and CW 5 are of the same size as those in other ovaries from non-pregnant animals.

Comrie & Adam note a gradual gradation in the sizes of the corpora albicantia in *Pseudorca*, which they suggest indicates that the whale is polyoestrous. Although not as many corpora albicantia are present in one ovary as in *Pseudorca* there are similar regular gradations of the size of the corpora albicantia in *Globiocephala*.

In *Pseudorca* there are no corpora albicantia present of the same size, except at the final stages. However, there are in *Globiocephala* several instances of an ovary containing corpora albicantia of approximately the same size and appearance, as in CW 23, 28, 29, 30 (Table 1). The latter contains three quite large corpora albicantia, arranged in a row on short peduncles. This finding does not necessarily indicate polyovulation, as corpora lutea in this condition do not all retrogress at the same rate. Twinning and multiple pregnancies have been reported in the Mystacoceti (Asdell, 1946), but in *Pseudorca*, only one foetus was found in each of seven specimens, and in *Globiocephala* only one foetus was found in each of three specimens. From this evidence and from the fact that the whales killed are at different stages in the reproductive cycle or pregnancy, it is probable that in *Globiocephala*, as in *Pseudorca*, ovulation is spontaneous and occurs several times a year.

It is well known that the accumulations of corpora albicantia in the ovary of the whalebone whales is an indication of their age (Mackintosh, 1946). The methods of estimation are, however, based on the assumption that the corpora albicantia never completely disappear. It has been suggested from the observations given that in *Globiocephala* the corpora albicantia do eventually retrogress to such a degree that, failing serially sectioning each ovary, no precise indication of the number of corpora albicantia in an adult ovary can be given. A further criticism of any method of determining the age of a whale is that there is at present no infallible method of telling if any corpus albicans is one of the cycle or of pregnancy. Comrie & Adam suggest that the finding of a regular gradation in the size of a series of corpora albicantia indicates a polyoestrous cycle; it may, however, equally well indicate a successive series of pregnancies. Thus CW 5 which contained at least one corpus luteum of pregnancy and four corpora albicantia in one ovary, and five corpora albicantia in the other, is either 4–6 years old, depending on the number of oestrous cycles in one year, or 21 years old, assuming that each corpus albicans represents a corpus luteum of pregnancy and that the whale becomes pregnant every 2 years (see Mackintosh, 1946; Comrie & Adam, 1938). There still remains the unknown number of corpora albicantia that are invisible to the naked eye. For this reason counting the number of corpora lutea is no indication of the age of any particular specimen of *adult Globiocephala*.

The presence of multinuclear luteal cells in the early corpora lutea and also in those of pregnancy, has not hitherto been described in any other mammal. Multinuclear luteal cells have, however, been previously illustrated, though no reference is made to them in the text. In Mackintosh & Wheeler's description of the ovary in the Blue and Fin whales large vacuolated multinuclear cells from the corpus luteum are illustrated in fig. 125 on p. 388. There is growing evidence that the granulosa cells in many animals, probably including man, do divide mitotically in the first few hours after ovulation. Furthermore Bullough (1946) has suggested that the mitotic divisions occurring in the granulosa cells of the mouse just before and just after ovulation are due to the mitogenic effects of oestrogen. In this connexion it is interesting to note that the oestrogen value of the liquor folliculi of the Blue whale, *Sibbaldus musculus*, has been found to be of the order of 2000 m.u./l. by Jacobsen (1935). This figure may be compared with those of 92–788 m.u./kg. of fluid from the follicles of the cow given by Parkes & Bellerby (1926).

It is therefore possible that the formation of multinuclear cells is associated with the relatively high content of oestrogen in the follicular fluid. The incomplete division of the luteal cells may possibly be associated with the large amounts of lipids that the luteal cells contain. The multinuclear cells appear at the time that vacuoles are found in ever increasing amounts in the luteal cells. However, a more likely factor is the relative anoxia to which the cells are subjected. At the time when the multinuclear cells are found vascularization of the large quantity of luteal tissue is only poorly developed. Thus the multinuclear conditions may well be the result of an unrestrained growth process possibly associated with high oestrogen content, occurring in a relatively anoxic environment. The presence of mitoses in the luteal cells in the retrogressing corpora lutea of *Sorex araneus* described by Brambell (1935) is probably due to some other factor, perhaps the removal of the stimulus (to the corpus luteum) of the L.H. from the anterior pituitary, or the relative increase in oestrogens derived from the placenta as pregnancy progresses.

SUMMARY

1. A general account is given of the anatomy of the reproductive tract in a foetus and in a number of adult specimens of *Globiocephala melaena*.

2. The changes found in the ovaries are described, and a brief account of follicular development and atresia is given.

3. Five recent corpora lutea are described; multinuclear luteal cells are present in four of the corpora lutea.

4. The luteal cells are derived from the granulosa cells of the follicle; they become heavily vacuolated early in their development.

5. The theca interna cells mostly remain at the periphery of the developing corpus luteum.

6. Corpora albicantia persist in the ovary for a long time. However, there is evidence that they eventually shrink so that they are invisible to the naked eye.

7. The corpora albicantia are thus of little use in estimating the precise age of an individual adult *Globiocephala*.

8. It is suggested that *G. melaena* is polyoestrous and that ovulation occurs spontaneously from one mature follicle at a time.

The author wishes to thank Prof. W. J. Hamilton for his interest and advice, and also Dr F. C. Fraser for his help on various points and for kindly allowing access to his valuable collection of references. Mr R. Watts and Mr R. J. McCulloch gave valued assistance with the difficult section-cutting and the photography is the work of Mr E. V. F. Pittock.

REFERENCES

Asdell, S. A. (1946). *Patterns of Mammalian Reproduction*. New York: Comstock Publ. Co.

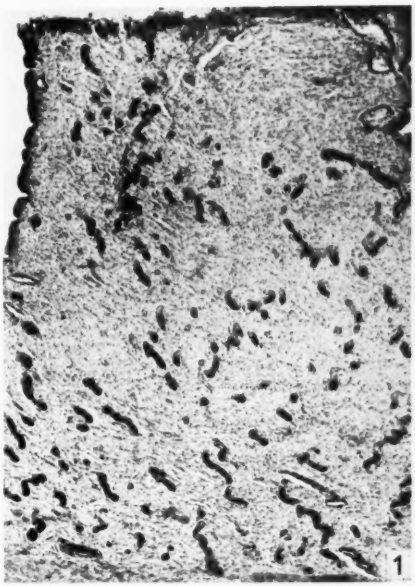
Brambell, F. W. R. (1935). Reproduction in the common shrew (*Sorex araneus* Linnaeus). *Philos. Trans.* B, **255**, 1–62.

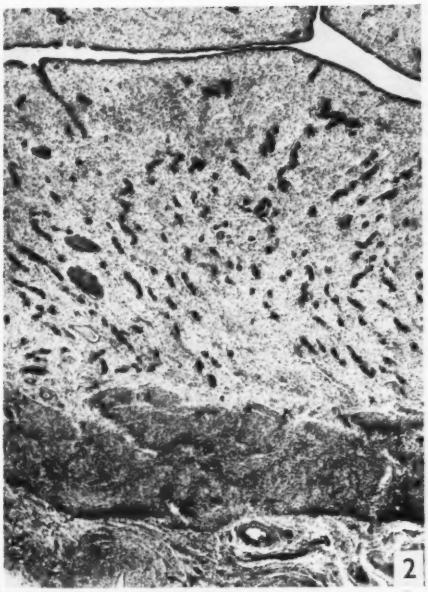
Bullough, W. S. (1946). Mitotic activity in the adult female mouse, *Mus musculus* L. *Philos. Trans.* B, **231**, 453–516.

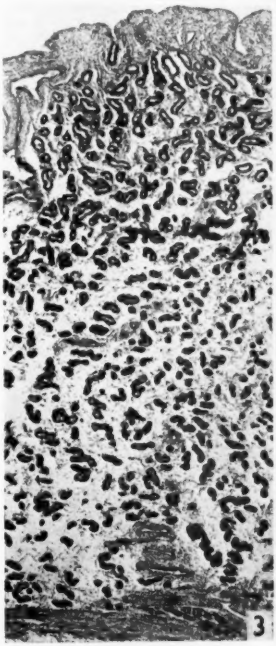
Comrie, L. C. & Adam, A. B. (1938). The female reproductive system and corpora lutea of the False Killer Whale, *Pseudorca crassidens* Owen. *Trans. Roy. Soc. Edinb.* **59**, 521–531.

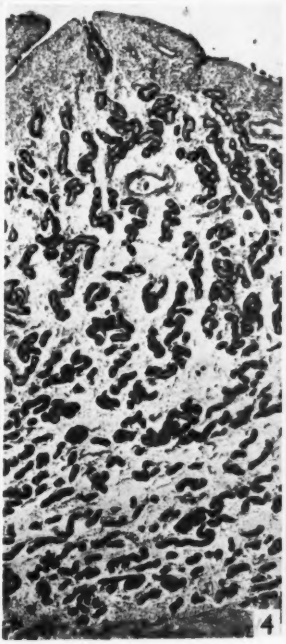
Daudt, W. (1898). Beiträge zur Kenntnis des urogenital Apparats der Cetacean. *Z. Naturw. Jena*, **32**, 231–312.

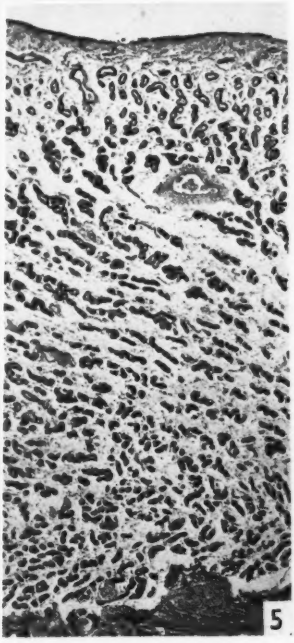
Harrison, R. J. (1946). The early development of the corpus luteum in the mare. *J. Anat., Lond.*, **80**, 160–166.

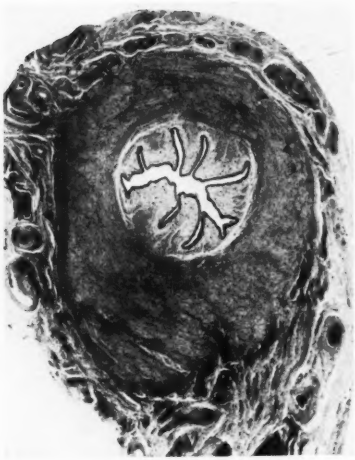




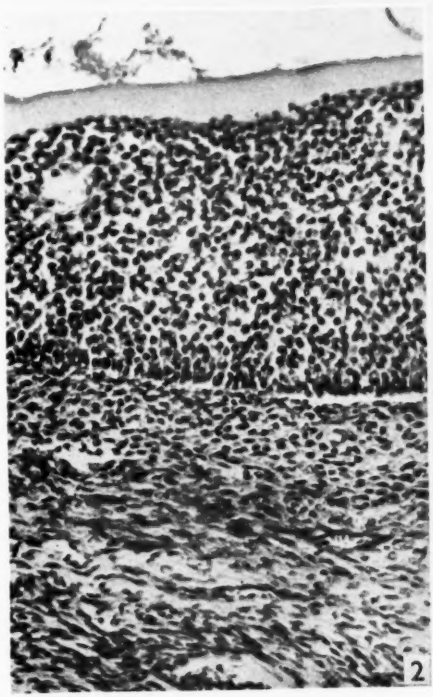




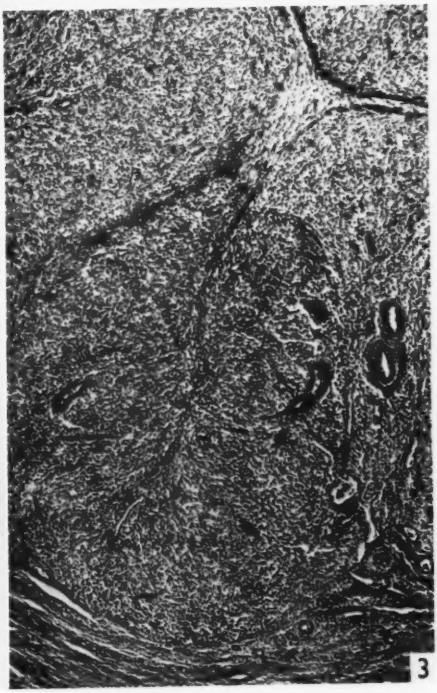




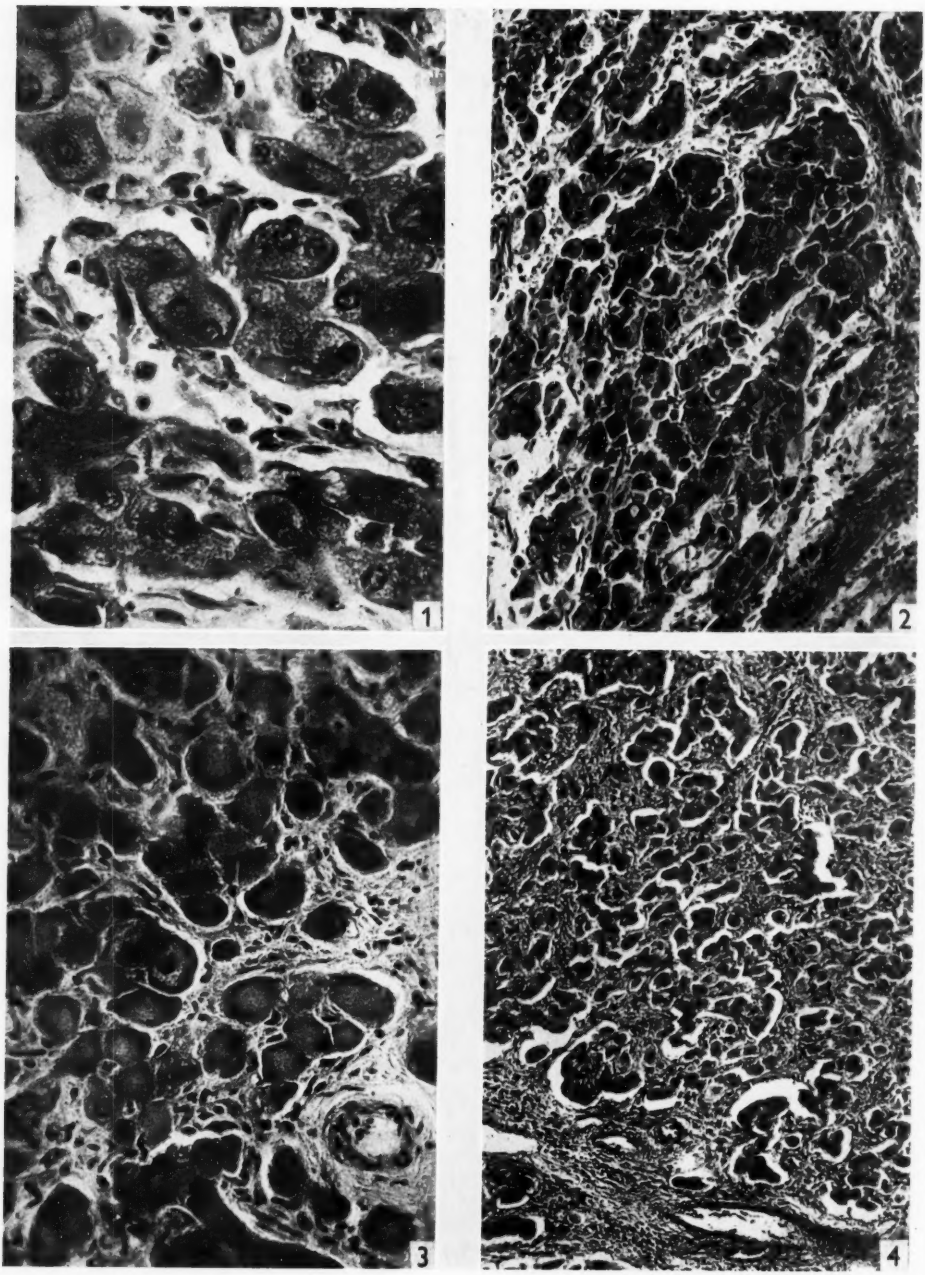
1



2



3



HARRISON—Reproductive organs of *Globiocephala*

HARRISON, R. J. (1948). The changes occurring in the ovary of the goat during the estrous cycle and in early pregnancy. *J. Anat., Lond.*, **82**, 21–48.

HUNTER, J. (1787). Observations on the structure and oeconomy of whales. *Philos. Trans.* **77**, 371–450.

JACOBSEN, A. P. (1935). Endocrine organs of the Blue Whale. *Nature, Lond.*, **136**, 1029.

KHVATOV, V. P. (1938). New information on the durability of retention of the corpus luteum in the Dolphin. *Bull. Biol. Med. Exp. U.R.S.S.* **55**, 27–88.

MACKINTOSH, N. A. (1946). The natural history of the whalebone whales. *Biol. Rev.* **21**, 60–74.

MACKINTOSH, N. A. & WHEELER, J. F. G. (1929). Southern blue and fin whales. '*Discovery*' *Rep.* **1**, 257–540.

MATTHEWS, L. H. (1948). Cyclic changes in the uterine mucosa of Balaenopterid whales. *J. Anat., Lond.*, **82**, 207–232.

MEEK, A. (1918). The reproductive organs of Cetacea. *J. Anat., Lond.*, **52**, 186–210.

MURIE, J. (1874). On the organization of Ca'aing whale, *Globiocephalus melas*. *Trans. Zool. Soc. Lond.* **8**, 235–301.

OMMANNEY, F. D. (1932). The urino-genital system of the fin whale (*Balaenoptera physalus*). '*Discovery*' *Rep.* **5**, 363–466.

PARKES, A. S. (1931). The reproductive processes of certain mammals. II. The size of the Graafian follicle at ovulation. *Proc. Roy. Soc.* B, **109**, 185–196.

PARKES, A. S. & BELLERBY, C. W. (1926). Studies on the internal secretions of the ovary. I. The distribution in the ovary of the oestrus-producing hormone. *J. Physiol.* **61**, 562–575.

PEARSON, O. P. & ENDERS, R. K. (1943). Ovulation, maturation and fertilization in the fox. *Anat. Rec.* **85**, 69–83.

PYCRAFT, W. P. (1932). The genital organs of a female common Dolphin (*Delphinus delphis*). *Proc. Zool. Soc. Lond.* pp. 807–811.

RITCHIE, J. (1924). Observations on a pilot whale stranded in the Firth of Forth. *Scot. Nat. Edinb.* pp. 37–43.

ROSSMAN, I. (1942). On the lipin and pigment in the corpus luteum of the rhesus monkey. *Contr. Embryol. Carneg. Instn*, **30**, 97–109.

STRASSMANN, E. O. (1941). The theca cone and its tropism toward the ovarian surface, a typical feature of growing human and mammalian follicles. *Amer. J. Obstet. Gynaec.* **41**, 363–378.

TURNER, W. (1867). A contribution to the anatomy of the Pilot Whale (*Globiocephalus* Svineval Lacépede) *J. Anat., Lond.*, **2**, 66–79.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Section through the body of the uterus of the anoestrous whale CW 10. Haematoxylin and eosin. ×35.

Fig. 2. Section through the uterine horn of the anoestrous whale CW 10. Haematoxylin and eosin. ×35.

Fig. 3. Section through the body of the uterus of the whale CW 11. An early corpus luteum is present in one ovary. Haematoxylin and eosin. ×20.

Fig. 4. Section through the fused uterine horns of the whale CW 11. Haematoxylin and eosin. ×20.

Fig. 5. Section through the uterine horn of the whale CW 11. Haematoxylin and eosin. ×20.

PLATE 2

Photographs of sections through the uterine tubes of the whales CW 10 (left) and CW 11. The magnification of both sections is ×13.

PLATE 3

Fig. 1. Photograph of the macroscopic appearances of the five recent corpora lutea. Scale in cm.

Fig. 2. Section through the wall of a maturing follicle 1 cm. in diameter. The theca interna layer is clearly seen. Haematoxylin and eosin. ×80.

Fig. 3. Low-power view of a section through the corpus luteum from whale CW 11. Haematoxylin and eosin. ×30.

PLATE 4

Fig. 1. High-power view of the luteal cells from the corpus luteum from whale CW 2. The multinuclear luteal cells can be seen. Haematoxylin and eosin. ×650.

Fig. 2. Section through the corpus luteum of CW 4. Haematoxylin and eosin. ×120.

Fig. 3. Section through the corpus luteum of CW 1. Haematoxylin and eosin. ×500.

Fig. 4. Section through the corpus luteum of CW 5. Haematoxylin and eosin. ×90.

A PRELIMINARY STUDY OF THE DISTRIBUTION OF CELL SIZE IN THE LATERAL GENICULATE BODY

By L. W. CHACKO (Madras)
From the Department of Human Anatomy, University of Oxford

INTRODUCTION

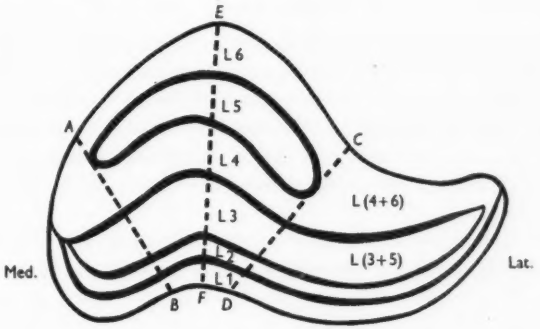
In the course of a comparative study of the laminar organization of the lateral geniculate body (Chacko, 1948*a*), a regular cell-size gradient was observed under the low-power microscope in the six-laminar central vision area as well as in the four-laminar peripheral vision area. It was decided to undertake a preliminary study of the spectrum of cell size, basing it on measurements of the size of the cells in the human geniculate nucleus in the various layers of both the central and peripheral vision projection areas. Supplementary data were obtained in a study of the same nucleus in the macaque monkey.

In studying the morphological details of the lateral geniculate body, it is apparent that the size characteristics of the cells lend themselves readily to quantitative measurements. The larger size and the more deeply staining character of the cells in layers 1 and 2 when compared with the cells in layers 3, 4, 5 and 6 have previously been recognized, and Balado & Franke (1937) have mentioned the approximate cell size in some of the layers. However, no systematic investigation of the mode of distribution of cell sizes has so far been undertaken. As regards the possible significance of such quantitative studies it should be recalled that Malone (1932) advanced the view that the size of a neuron is related to its specific function, to the volume of its activity, and to its tendency to maintain a uniform irritability. Although the central problem of cyto-architecture, namely, its functional significance, is far from solved, additional information along structural lines also may prove to be of value.

MATERIAL AND METHODS

A block of human brain tissue containing the lateral geniculate body was cut in 10 μ sections in a plane at right angles to the course of the optic tract and stained with Borell's methylene blue. The regions of the nucleus chosen for making measurements were selected in terms of the various retinal projection areas. In the central vision area which is, in general, characterized by six laminae, four antero-posterior levels were chosen at regular intervals from the foveal centre towards the periphery (cf. Sections A, B, C and D in Table 1). In the human brain, when these six laminae are traced to the peripheral vision area, layers 1 and 2 are continued as separate and discrete layers, whereas layer 3 fuses with layer 5, and layer 4 with layer 6 to form two 'small-celled' laminae (Text-fig. 1). These two composite layers of the peripheral vision area will be designated in the following as layers (3+5) and (4+6). In the projection area for the lower homonymous quadrants of the peripheral hemiretinae four antero-posterior levels were chosen, and in that for the upper homonymous quadrants three levels were chosen for measurements of the cells of their respective four laminae (cf. Table 1).

As was shown in a previous study (Chacko, 1948*a*), the central vision area has the shape of an inverted pyramid with a flattened apex directed towards the ventral hilum and a convex base directed towards the dorsal crest (Text-fig. 1). In frontal sections this region appears roughly triangular, *EF* representing the axial plane corresponding to the horizontal meridian of the retina. The sample fields in each of the layers of the central vision area were located in a strip extending along the axial plane from the hilum to the dorsal crest. Since layers 1 and 2 (the large-celled elements) are very thin, less dense than the other layers, and limited in extent in this region, only 25–40 cells were found to be available for measurements in the sample fields of these laminae. In the case of layers 3, 4, 5 and 6 (the small-celled elements) 60 cells were measured in each layer of each of the four sections studied so that a total of 240 cells were measured in each layer, amounting to 960 cells in the whole central vision area.



Text-fig. 1. Diagram illustrating the extent of the central and peripheral vision projection areas in a frontal section through the human lateral geniculate body. *AB* indicates boundary between central vision area and the projection area for the upper homonymous quadrants of the peripheral hemi-retinae (medial tubercle). *CD* indicates boundary between central vision area and the projection area for the lower homonymous quadrants of the peripheral hemi-retinae (lateral horn). *EF* represents the axial plane corresponding to the horizontal meridian of the retina. Lat. lateral. Med. medial.

The 'lateral horn', which represents the projection area for the lower homonymous quadrants of the hemi-retinae, exhibits a four-laminar pattern throughout its extent. The sample fields in this region were located along a strip midway between the free margin of the 'lateral horn' and its junction with the central vision area. In the projection area for the upper homonymous quadrants, only the medial tubercle shows the typical four-laminar pattern and the measurements were, therefore, limited to the cells of the tubercle. Because this region is relatively limited in extent in any single section, the strips chosen for measurements covered nearly the whole distance between the central vision area and the medial end of the tubercle. In both of the peripheral vision projection areas, the number of cells available for measurements in layers 1 and 2 was somewhat limited, although not to such an extent as in the central vision area. In most of the sections the number of cells measured represented the total number available in each section. The number of cells measured in each of layers 1 and 2 ranged from 18 to 47. In each of the composite layers

(3+5) and (4+6), 40–60 cells were measured. The monocular vision area was not included in the present study.

The central vision area of the geniculate nucleus of the macaque monkey was studied briefly in one typical section (Table 2).

In view of the fact that nerve cells may assume very irregular shapes, it is rather difficult to make precise measurements of their size. The cells of the human geniculate nucleus, as is evident from Pl. 1, appear triangular, polygonal, spindle- or flask-shaped. The method finally adopted for measurements consisted in measuring the long and the short diameters of the cell bodies by means of a screw eyepiece micrometer. The criterion on the basis of which cells were selected for measurements consisted in choosing only cells with intact and well-defined nucleoli. For further statistical studies the mean of the large and small diameters was noted for each cell.

RESULTS

The results of the measurements of the diameters of cells in various projection areas of the human geniculate nucleus are summarized in Table 1. First, the results obtained in measuring the cells of the central vision area will be considered. An inspection of the microphotograph in Pl. 1 shows not only the marked size differences between the cells of laminae 1 and 2 on the one hand and laminae 3–6 on the other, but also seems to indicate a size gradient from lamina 3 to 6. Such an impression is

Table 1. *Means and standard deviations of diameters of cells in μ measured in different laminae of various projection areas in the human lateral geniculate body*

Layer	No. of cells measured	Mean	σ
		Central vision area	
Section A			
1	40	22·0±0·57	3·61±0·40
2	25	24·8±0·65	3·24±0·46
3	60	19·1±0·31	2·41±0·22
4	60	15·6±0·22	1·74±0·16
5	60	15·4±0·25	1·96±0·18
6	60	14·2±0·24	1·89±0·17
Section B			
1	30	23·9±0·38	2·09±0·27
2	28	24·5±0·61	3·25±0·43
3	60	18·9±0·27	2·12±0·19
4	60	17·8±0·31	2·37±0·22
5	60	15·8±0·20	1·54±0·14
6	60	13·8±0·25	1·95±0·18
Section C			
1	30	22·8±0·53	2·89±0·37
2	30	23·0±0·57	3·11±0·40
3	60	18·2±0·34	2·65±0·24
4	60	17·0±0·20	1·56±0·14
5	60	15·5±0·29	2·28±0·21
6	60	14·8±0·21	1·60±0·15
Section D			
1	30	23·6±0·62	3·40±0·44
2	30	23·8±0·58	3·19±0·41
3	60	19·5±0·25	1·96±0·18
4	60	17·9±0·29	2·22±0·20
5	60	16·9±0·31	2·40±0·22
6	60	14·7±0·24	1·87±0·17

Table 1 (*continued*)

Layer	No. of cells measured	Mean	σ
Projection area for the lower homonymous quadrants of the peripheral hemi-retinae			
Section A			
1	30	21·7 ± 0·54	2·98 ± 0·38
2	18	22·8 ± 0·61	2·62 ± 0·44
(3 + 5)	60	17·5 ± 0·22	1·74 ± 0·16
(4 + 6)	60	16·8 ± 0·22	1·72 ± 0·16
Section B			
1	47	21·3 ± 0·47	3·26 ± 0·34
2	29	23·5 ± 0·57	3·07 ± 0·40
(3 + 5)	60	18·3 ± 0·28	2·18 ± 0·20
(4 + 6)	60	15·8 ± 0·20	1·53 ± 0·14
Section C			
1	44	20·4 ± 0·40	2·65 ± 0·28
2	32	21·2 ± 0·55	3·12 ± 0·39
(3 + 5)	60	17·8 ± 0·24	1·89 ± 0·17
(4 + 6)	60	15·4 ± 0·19	1·52 ± 0·14
Section D			
1	22	23·6 ± 0·73	3·43 ± 0·73
2	38	23·5 ± 0·39	2·39 ± 0·27
(3 + 5)	60	18·9 ± 0·28	2·17 ± 0·20
(4 + 6)	60	16·2 ± 0·24	1·88 ± 0·17
Projection area for the upper homonymous quadrants of the peripheral hemi-retinae			
Section A			
1	20	23·6 ± 0·42	1·92 ± 0·30
2	20	21·4 ± 0·51	2·27 ± 0·36
(3 + 5)	40	16·7 ± 0·32	2·01 ± 0·22
(4 + 6)	60	15·5 ± 0·22	1·67 ± 0·15
Section B			
1	47	23·4 ± 0·47	2·99 ± 0·33
2	29	23·7 ± 0·54	2·43 ± 0·38
(3 + 5)	60	17·9 ± 0·28	2·15 ± 0·20
(4 + 6)	60	16·1 ± 0·25	1·92 ± 0·17
Section C			
1	40	23·0 ± 0·41	2·60 ± 0·29
2	20	25·8 ± 0·37	1·64 ± 0·26
(3 + 5)	60	17·0 ± 0·21	1·62 ± 0·15
(4 + 6)	60	16·8 ± 0·29	2·27 ± 0·21

confirmed by the data presented in Table 1. It is seen that there is a decrease in the mean diameters of the cells in the ventro-dorsal direction from lamina 3 to 6 in all the sections studied. Further analysis indicates that the differences between the means of the cell diameters in the small-celled laminae 3–6 are, in practically all instances, statistically significant. The fact that the cells decrease significantly in size in the ventro-dorsal direction is also evident from the histograms of laminae 3–6 in Text-fig. 2 and from the smoothed frequency polygons in Text-fig. 4.

As regards the results obtained in measuring the cells of the projection areas for the lower and upper homonymous quadrants of the peripheral hemi-retinae, the findings are similar to those in the central vision area in that there exists a size gradient in the small-celled layers (3 + 5) and (4 + 6). There is a decrease in the mean diameters of the cells in the ventro-dorsal direction from one lamina to the other (Table 1). The mean diameter of the cells in lamina (4 + 6) is significantly smaller than that of the cells in lamina (3 + 5) in six out of the seven sections studied. The histograms in Text-fig. 3 and the smoothed frequency polygons in Text-fig. 5 illustrate this decrease in size with reference to the projection area for the lower homonymous quadrants of the peripheral hemi-retinae.

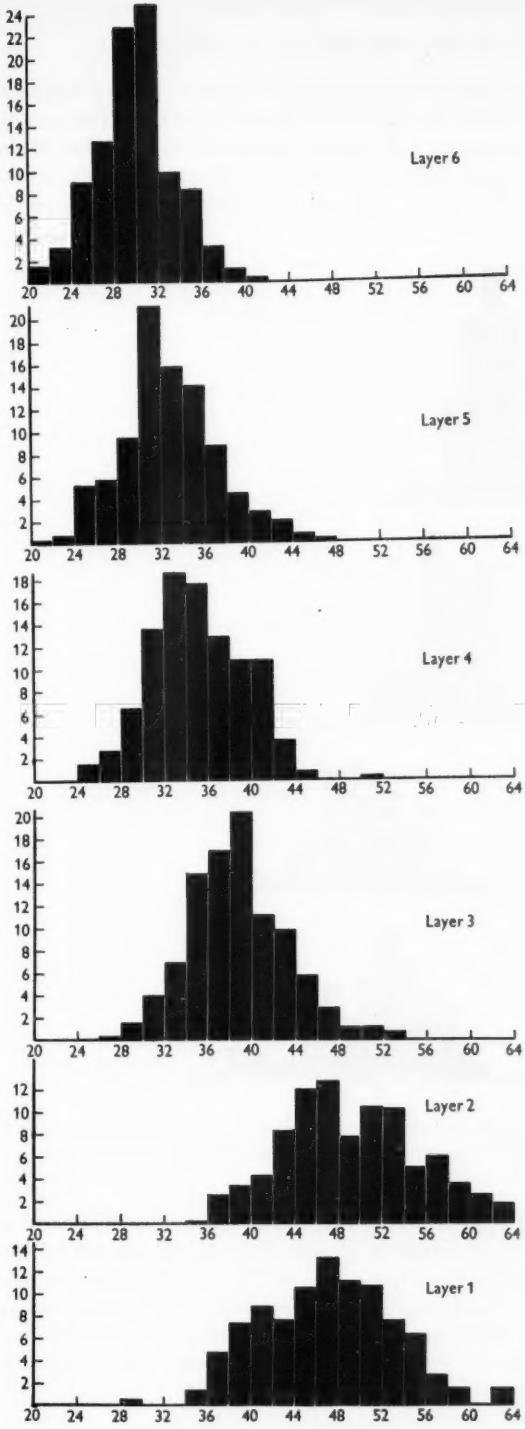
An analysis of the measurements in the sample fields of the large-celled laminae 1 and 2 does not reveal a size gradient as definite as that found in the small-celled laminae. As regards the central vision area, the mean diameter of the cells in lamina 2 is larger than that of the cells in lamina 1, but only in one of the four sections studied is the difference between the means statistically significant (Table 1; cf. also Text-figs. 2 and 4). In considering the measurements in the projection area for the lower homonymous quadrants of the peripheral hemi-retinae it is evident from Table 1 that the mean diameter of the cells in lamina 2 is larger than that of the cells in lamina 1 in three of the four sections studied. This difference is found to be at least tolerably significant (cf. also Text-figs. 3 and 5). In the projection area for the upper homonymous quadrants of the peripheral hemi-retinae the mean diameter of the cells in lamina 2 is found to be larger than that of the cells in lamina 1 in two of the three sections studied, but in only one is the difference statistically significant. It must be remembered that the laminar organization of this region is not as uniform as in the projection area for the lower homonymous quadrants, and that the medial tubercle presents a limited and rather unsatisfactory area for making measurements. If the data on cell diameters in the large-celled laminae of the central and peripheral vision areas are viewed as a whole, it becomes apparent that they have failed to establish any clear-cut size gradient.

In considering the transition from the large-celled to the small-celled layers, that is, the difference between laminae 2 and 3 in the central vision area and that between 2 and (3+5) in the peripheral vision areas, it is evident that the mean diameter of the cells in lamina 2 tends to be markedly larger in all the sections studied (Table 1). In all instances the differences between the means of laminae 2 and 3 or between 2 and (3+5) are statistically significant.

An inspection of the histograms in Text-figs. 2 and 3 and of the smoothed frequency polygons in Text-figs. 4 and 5 clearly indicates that the several laminae differ in variability or dispersion of measurements. The range of cell diameters measured in the dorsal laminae is definitely smaller than that in the ventral laminae.

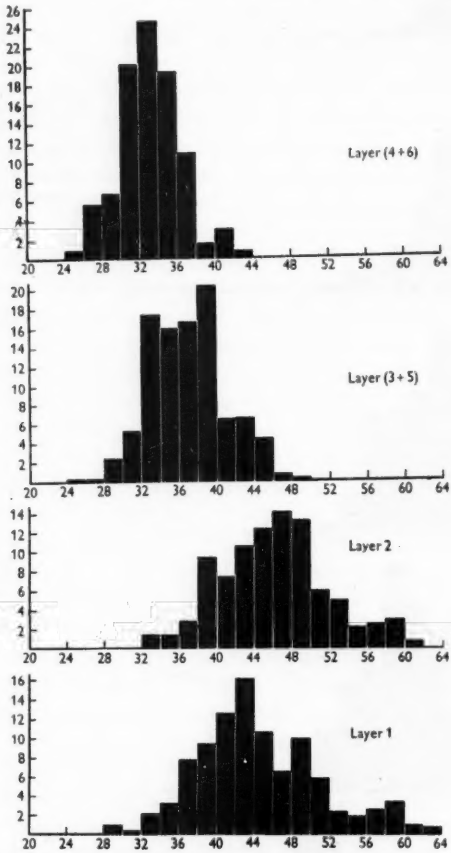
An analysis of the results with reference to crossed and uncrossed laminae readily shows the significant differences which exist in the central vision area between the mean diameters of the cells in layers 1 and 4 and layers 4 and 6 as well as between the mean diameters of the cells in layers 2 and 3 and layers 3 and 5. Similarly, significant differences exist between the means of the crossed and uncrossed layers of the peripheral vision area, that is, between layers 1 and (4+6) as well as between layers 2 and (3+5). Since in Text-figs. 4 and 5 the frequency distribution curves for the crossed laminae have been indicated by solid lines in contrast to the dotted lines for the uncrossed laminae, it can be seen at a glance that the curves for the crossed laminae are displaced towards the shorter diameters. This displacement is found in both central and peripheral vision areas and is especially marked in the small-celled layers. In Text-fig. 4, although there is an overlapping of the curves, L6 is located more to the left than L5, L4 more to the left than L3, and L1 more to the left than L2. In Text-fig. 5, L(4+6) is located more to the left than L(3+5), and L1 more to the left than L2.

If the means of the cell diameters of the various laminae of the central and peripheral vision areas are compared (Table 1), it is found that the laminae with the



Text-fig. 2. Frequency of diameters of cells in laminae 1–6 in central vision area. Abscissa: diameter in μ ($\times 2$). Ordinate: percentage frequency.

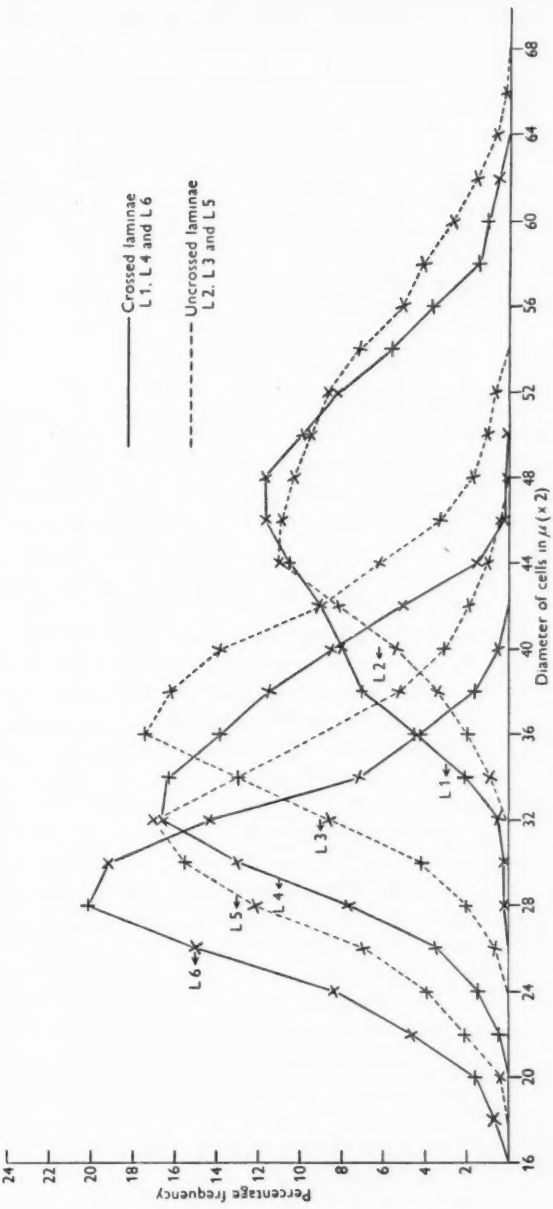
smallest mean cell diameters are located in the central vision area. There is a greater range of cell diameters in the central than in the peripheral vision area. This greater range is brought about by the occurrence of smaller cell diameters. Further, if the composite laminae of the peripheral vision area and their daughter laminae in the central vision area into which the former split, are compared, the laminae of the



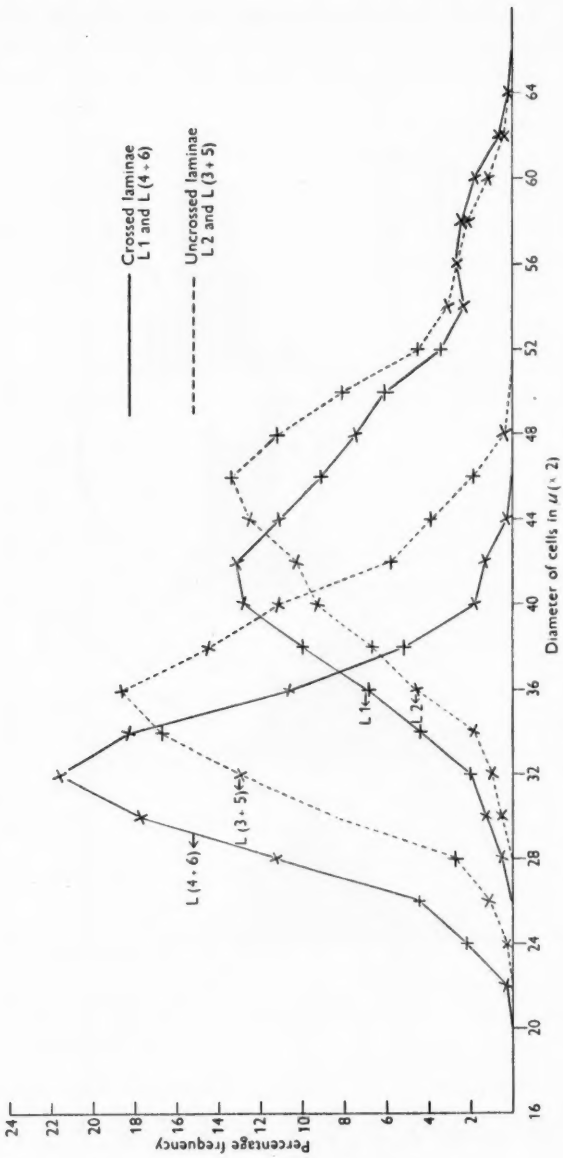
Text-fig. 3. Frequency of diameters of cells in laminae 1, 2, (3+5) and (4+6) in the projection area for the lower homonymous quadrants of the peripheral hemi-retinae. Abscissa: diameter in μ ($\times 2$). Ordinate: percentage frequency.

central vision area are found to have a greater range of cell diameters. The mean of the distribution of all cell diameters measured in each of the parent laminae of both the peripheral vision areas lies between the means of the two daughter laminae. Similarly, the median of the distribution of the cell diameters of each parent lamina lies between the medians of the distributions of the corresponding daughter laminae.

From a comparative point of view it is of special interest that a cell-size gradient



Text-fig. 4. Frequency of diameters of cells in different laminae of the central vision area.



Text-fig. 5. Frequency of diameters of cells in the different laminae of the projection area for the lower homonymous quadrants of the peripheral hemi-retinae.

similar to that in the human geniculate nucleus is also present in the lateral geniculate body of the rhesus monkey (Pl. 2 and Table 2).

Table 2. *Means and standard deviations of diameters of cells in μ measured in different laminae of the central vision area in the lateral geniculate body of the rhesus monkey*

Layer	No. of cells measured	Mean	σ
1	28	23·5±0·47	2·47±0·33
2	28	24·5±0·65	3·20±0·43
3	28	17·6±0·40	2·12±0·28
4	28	15·3±0·36	1·90±0·25
5	28	14·9±0·30	1·57±0·21
6	28	14·5±0·37	1·97±0·26

DISCUSSION

It has been suggested by several investigators, but principally by Malone (1932), that the functional specificity of nerve cells is related to their internal structure, their shape, and their size. Malone has made an extensive study of the fundamental types of nerve cell structure. He emphasizes the point that the cell bodies and dendrites have a higher metabolic rate and exhibit a greater variation in histological character than axons, and assumes that these features are associated with a wide range of local activity radically different from that of the axons. According to him, the size of the cell body and dendrites is dependent on the activity of the neuron and the extent to which it receives stimuli. He points out, for instance, that typical correlating neurons have small cell bodies with extensively branched axons and poorly developed dendrites and, furthermore, that they are numerous and lie close together in centres in which impulses diffuse widely and do not enter into definite common paths in contrast to co-ordinating centres containing relatively few neurons with large cell bodies and well-developed dendrites. In his opinion, the resistance to fatigue varies with the size of the cell body, the small cell bodies of typical correlating centres fatiguing more easily than large cell bodies.

The present study has established the existence of a size gradient in the central and peripheral vision areas of the lateral geniculate body. The measurements indicate that the range of cell diameters is smaller in the peripheral than in the central vision area. It has also been found that each of the layers (3+5) and (4+6), when traced to the central vision area, splits into two laminae and that the means (as well as the medians) of the frequency distributions of the two daughter laminae are larger or smaller than the mean (or median) of the parent lamina.

In speculating on the possible significance of these findings, the attempt may be made to interpret the activity of the laminae as follows: a lamina possesses a number of neurons comprising a certain range of sizes and yielding a typical frequency distribution. These elements acting as a whole are capable of producing a certain type of total activity. The resultant activity of any particular lamina will be chiefly determined by the activity of its middle range elements, but such activity will probably be modulated by the elements at the two extremes of the distribution. The splitting of a particular lamina into two discrete laminae will widen the range

of activity within which each daughter lamina may then be assumed to establish its own specific maximum activity, and the latter is directly determined by its middle-range elements. Various findings in sensory physiology and psychology clearly indicate that there exist numerous visual functions with respect to which the discriminative capacities of the central retina are superior to those of the peripheral retina.

It has been previously pointed out in Text-figs. 4 and 5 that the distribution curves for the crossed laminae **L1, L4** and **L6** are displaced towards the shorter diameters when compared with the distribution curves for the uncrossed laminae **L2, L3** and **L5**. Differently expressed, the cells of the uncrossed laminae are on the whole relatively larger than those of the crossed laminae. The laminae related to the corresponding halves of the two eyes are identical in number, but they are not necessarily duplicate mechanisms in functional respects since they are not identical morphologically. It appears likely, therefore, that the neural elements related to one eye respond to a certain extent differently from those related to the other eye whenever corresponding points of the retinae are simultaneously stimulated. The impulses from the two corresponding points are relayed to the same 'unit' in the geniculate body although it must be remembered that the crossed laminae of such a 'unit' are, as enucleation experiments indicate, morphologically independent of the uncrossed laminae. Anatomical considerations of this nature thus lead to the assumption of certain functional differences between temporal and nasal halves of the retina, but they do not suggest that such differences are very pronounced. It is to be expected that the functional efficiency of either the temporal or nasal half of one retina is less than that of two homonymous hemi-retinae and that the impulses arriving from the contralateral eye lead quantitatively and qualitatively to a widening of the response activity. The functional superiority of the nasal retina in visual acuity, colour sensitivity, etc., has been commented on by various investigators (Köllner, 1920; Best, 1917; Gelb & Goldstein, 1925; Klüver, 1927). It is Köllner's thesis that there exists an inequality of 'corresponding impressions' and a superiority of the nasal retina. The data of the present investigation do not suggest a functional superiority of either the nasal or temporal half of the retina, but they do seem to provide an anatomical basis for an inequality of 'corresponding impressions'. Since the nasal as well as the temporal retina, each in its own specific way, contributes to binocular vision, the conducting units of fibres from both eyes and the total number of laminae in each projection area will have to be taken into consideration in analysing 'units' in the visual system.

The existence of a size gradient in the central and peripheral vision areas of the lateral geniculate body is of particular interest in view of the existence of various morphological gradients in other parts of the visual system. In the receptor layer of the retina, the rods slowly and gradually increase in thickness and decrease in length from the central area to the periphery. Similarly, the cones change from short and thick to long and fine structures, thus exhibiting a gradient in regard to both size and shape. An investigation of the different varieties of bipolar and ganglion cells likewise yields size gradients. It has been observed that the axons and telodendria of the larger retinal neurons are thicker than those of the smaller neurons (Polyak, 1941). A study of the optic nerve reveals a continuous fibre

spectrum (Chacko, 1948*b*). As regards the optic radiation, Polyak mentions that the calibre of the fibres varies from fine to medium and coarse fibres.

If the assumption is made that cell size is one of the morphological characteristics related to functional specificity, cell-size gradients, such as exist in the geniculate body as well as in various layers of the retina, may be thought to have some relation to functional gradients of various kinds. Gasser and his collaborators have, for instance, established an approximately linear relationship between the velocity of conduction and the diameter of nerve axons (Gasser, 1941). As regards the visual system, it mediates responses to differences in radiation varying in luminous intensity or in wave-length. The responses made are frequently responses to stimuli of a graded character. The organism must be able to fall back on mechanisms capable of dealing with stimuli lying in the same 'dimension', such as neutral colours constituting a brightness series or the variations in hue in certain portions of the spectrum. The question may be raised whether cell size gradients or fibre gradients are related to or suggestive of mechanisms which cope with variations in the 'quantity' or 'quality' of light. The problem also arises whether mechanisms, such as Granit's modulators (1947) yielding narrow sensitivity curves in different regions of the spectrum, have some counterpart in the anatomical organization of the lateral geniculate body. Neither the histological nor the electrophysiological analysis has reached the point at which profitable hypotheses can be offered. In the meantime, Hartridge (1948) is undoubtedly right in pointing out that Granit's modulators form 'what amounts to a polychromatic series'. Further research will be necessary to determine whether the serial character of the modulators is in some way related to size gradients or other morphological gradients in the visual sector of the central nervous system.

I wish to thank Prof. Le Gros Clark for the facilities of his department to carry out this work and Prof. Heinrich Klüver for a critical discussion of the subject and for help in the preparation of the manuscript.

REFERENCES

Balado, M. & Franke, E. (1937). *Das Corpus Geniculatum Externum.* Berlin: J. Springer.

Best, F. (1917). Hemianopsie und Seelenblindheit bei Hirnverletzungen. *v. Graefes Arch. Ophthal.* **93**, 49–150.

Chacko, L. W. (1948*a*). The laminar pattern of the lateral geniculate body in the primates. *J. Neurol. Neurosurg. and Psychiat.* **11**, 211–224.

Chacko, L. W. (1948*b*). An analysis of fibre-size in the human optic nerve. *Brit. J. Ophthal.* **32**, 457–461.

Gasser, H. S. (1941). The classification of nerve fibres. *Ohio J. Sci.* **41**, 145–159.

Gelb, A. & Goldstein, K. (1925). Zur Frage nach der gegenseitigen funktionellen Beziehung der geschädigten und der ungeschädigten Sehsphäre bei Hemianopsie (Mikropsie infolge der Vorherrschaft der Vorgänge in der geschädigten Sehsphäre). *Psychol. Forsch.* **6**, 187–199.

Granit, R. (1947). *Sensory Mechanisms of the Retina.* Oxford University Press.

Hartridge, H. (1948). Recent advances in colour vision. *Science*, **108**, 395–404.

Klüver, H. (1927). Visual disturbances after cerebral lesions. *Psychol. Bull.* **24**, 316–358.

Köllner, H. (1920). Das gesetzmässige Verhalten der Richtungslokalisation im peripheren Sehen nebst Bemerkungen über die klinische Bedeutung ihrer Prüfung. *Pflüg. Arch. ges. Physiol.* **184**, 134–155.

Malone, E. F. (1932). The general relation of histological character to function in mammalian neurons. *Spec. Cytology*, ed. by E. V. Cowdry, vol. **3**, 1403–1420. New York: Hoeber and Co.

Polyak, S. (1941). *The Retina.* Chicago: University of Chicago Press.

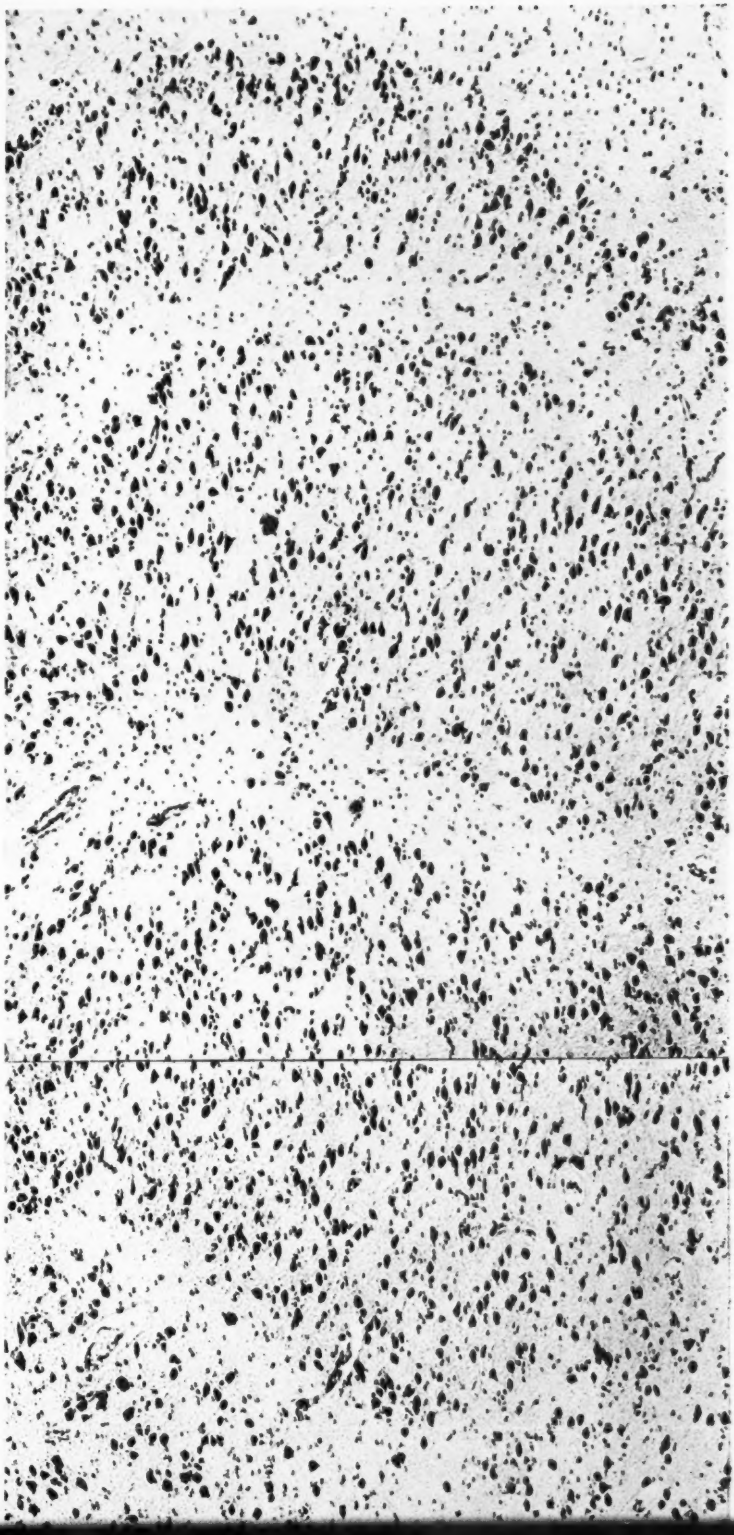
EXPLANATION OF PLATES

Plate 1

Microphotograph of a section through the central vision area of the human lateral geniculate body. ×80.

Plate 2

Microphotograph of a section through the small-celled laminae of the central vision area in the lateral geniculate body of the rhesus monkey. ×80.



CHACKO—DISTRIBUTION OF CELL SIZE IN THE LATERAL GENICULATE BODY

THE DISTRIBUTION OF THE VASAL AND CREMASTERIC ARTERIES TO THE TESTIS AND THEIR FUNCTIONAL IMPORTANCE

By R. G. HARRISON

From the Department of Human Anatomy, University of Oxford

In a previous study of the vascularization of the human testis (Harrison & Barclay, 1948) attention was called to the distribution of the vasal (deferential) and cremasteric arteries, and the manner of their anastomoses with the testicular artery, and to the importance of these in relation to deliberate or inadvertent ligature of the testicular artery in surgical operations such as orchiopexy, herniorrhaphy and radical operation for varicocoele. Although many observers have noted the effects of ligature or division of the testicular artery in experimental animals, it has not been possible to find any reference in the literature to work considering these effects in relation to the site of the anastomoses which the testicular artery makes with the vasal and cremasteric arteries. The present communication is concerned first with an experimental study of the effects on the rat testis of interruption of the testicular artery at definite points before and after its anastomosis with the vasal artery and, secondly, the manner of anastomosis of the vasal and cremasteric arteries with the testicular artery in Man as demonstrated by arteriography. Finally, consideration is given to the application of the experimental results to the problems of ligature of the testicular artery in Man.

EXPERIMENTAL RESULTS OF LIGATURE OF THE TESTICULAR ARTERY

Historical résumé

Cooper (1830) observed that division of the testicular artery and vein in a dog was followed by gangrene of the testis. Miflet (1879) embolized the testicular artery, or ligated the testicular artery alone, or the testicular artery and veins, or merely the testicular veins, in the dog. He observed the effects on the testis for periods up to 6 months, and found that any obstruction to the blood flow through the testicular artery led to a haemorrhagic infarct of the testis; as early as 48 hr. after interruption of the blood flow to the testis the cell content of the seminiferous tubules had coalesced to an almost homogeneous mass, and the interstitial tissue showed degenerative changes. Miflet concluded that the testicular artery has for the testis the significance of an end-artery in Cohnheim's sense. Enderlen (1896) claimed that a ligature left around the spermatic cord in the dog for periods up to 16 hr. caused no damage to the testis; periods longer than this led to atrophic changes. Griffiths (1896) found that after ligature of the testicular artery alone in dogs the testis showed great decrease in size in a few days, due to rapid destruction from degenerative changes in the seminiferous tubules; at later periods, particularly in one testis 3 months after operation, he found normal seminiferous tubules, which he concluded to be in process

of regeneration. Ligature of the testicular veins alone caused haemorrhagic infarction of the testis; ligature of the testicular artery and veins together eventually led to complete atrophy of the testis, except in one adult dog 130 days after operation in which the ligature could not be found and the testicular artery had established free communication again. Griffiths assumed from these results that the germinal epithelium can recover from a temporary degeneration and resume spermatogenesis. In experiments performed also on dogs, Capurro (1902) claimed that there are profuse anastomoses between the testicular and vasal arteries (fundamental arteries) and the cremasteric and external and internal pudendal arteries (accessory arteries). Even if all of these arteries are ligated on one side he claimed that an injection mass could pass to the testis by way of anastomoses with the other side. Schinz & Slotopolsky (1924), in their monograph on the experimental pathology of the testis, describe several experiments made on guinea-pigs and rabbits in which the testicular vessels were divided or compressed by scar tissue high up in the abdominal cavity following operations for the extirpation of the lumbar sympathetic chain, or when these vessels were directly ligated or sectioned; hypoplasia, 'regressive metamorphosis', necrosis or atrophy always resulted. They warn against the interpretation of results following ligature and not section of the testicular vessels. Section of the testicular artery only, according to Wangensteen (1927), is always followed by diminution in size of the dog's testis and scattered degenerative changes in the tubules, spermatogenesis still continuing. Ligature of the testicular artery and all the veins in the pampiniform plexus always results in total destruction of the testis, the latter becoming transformed into fibrous tissue. Ligature for 10 hr., and subsequent release, of all the structures in the spermatic cord of dogs and rabbits produces complete degeneration and fibrosis after 45 days, according to Iwasita (1939); this degeneration does not occur after only 5 hr. compression.

In most of the above investigations no definite indication is given as to the exact point of ligature, or section of the testicular vessels, in relation to the anastomoses which the artery makes with other vessels. However, most observers are in agreement in finding that ligature of the testicular artery and vein is followed by degenerative changes in the testis. The present investigation is concerned with an examination of the effects of interruption of the testicular artery in the rat in specific relation to the site of anastomoses which this artery makes with the vasal artery.

The vascular pattern of the rat's testis compared with that of the dog

Before undertaking an experimental study of the effects of interruption of the blood supply of the testis in the rat, it was necessary to examine by means of arteriography the vascular pattern and anastomotic connexions in the normal animal. A parallel study was made of the vascularization of the dog's testis for the reason that, as already indicated, almost all the previous investigations on the effect of interruption of the blood supply of the testis have been carried out on this species.

'Chlorbismol' (May and Baker Ltd.), a suspension of bismuth oxychloride in 5 % dextrose solution, was used as the radiopaque medium throughout this investigation in preference to colloidal bismuth, for while 'Chlorbismol' in 10–20 % suspension has particles of a more or less uniform size (10–12 μ), the particles of colloidal metallic bismuth in 5 or 10 % suspension have the tendency to aggregate to form

a suspension containing particles of unequal size and as much as 28μ in diameter. Hence, while both of these injection media will display only channels which are presumably functional and greater than capillary size, there is a tendency for colloidal bismuth not to fill certain channels due to blockage with the larger-sized particles. 'Chlorbismol', however, will fill the whole vascular bed of an organ, excluding veins and capillaries, quite evenly when injected through its artery, and display subsequently a very clear radiograph. All radiography was done on 'Kodaline' (Kodak nitrate) slow film using a micro-radiography apparatus (Barclay, 1947).

The arterial supply of the rat's testis, as demonstrated by radiography after the injection of 'Chlorbismol' into the thoracic aorta, is shown in Pl. 1, fig. 1. The testicular artery undergoes convolutions before reaching the testis and first gives off a branch to the fatty body; the epididymis is vascularized either by two separate branches for caput and cauda epididymidis, or, as in Pl. 1, fig. 1, by a single stem which divides into these two branches. These two vessels always anastomose by a junctional vessel running along the body of the epididymis, and the branch to the cauda epididymidis anastomoses with the vasal artery. The vasal artery may be seen in Pl. 1, fig. 1, to give off a small tortuous vessel which passes on to the scrotum into the territory of termination of the cremasteric artery. Neither by close examination of the radiographs nor by dissection, however, could there be demonstrated any continuity of the injection mass in the vasal and cremasteric arteries. After giving off its branches to the epididymis the testicular artery appears to be an end-artery, and, after curving around the inferior pole of the testis, convolutes before passing into the testis near its superior pole. Once inside the testis this artery gives off its first branches to the testis parenchyma in its course towards the inferior pole; in this part of its course the artery is lying near the epididymal border of the testis. In no testis out of more than thirty examined was the cremasteric artery filled by retrograde injection after injecting 'Chlorbismol' through the testicular artery.

The appearance of the arterial supply of the dog's testis, as seen by radiograph after the injection of 'Chlorbismol' in six testes at a pressure of approximately 100 mm. mercury, is shown in Pl. 1, fig. 2. On approaching the testis the testicular artery forms a number of convolutions. Before doing so, however, the artery gives off a branch which vascularizes the caput epididymidis. A further branch to the cauda epididymidis is given off from the convoluted part of the testicular artery, which passes along the length of the epididymis to the cauda and here anastomoses with the vasal artery. There are no anastomoses between the testicular artery at the inferior pole of the testis and the vessels on the cauda epididymidis, so that the testicular artery, after giving off its branch to the cauda is an end-artery as far as the testis is concerned. The cremasteric artery is not filled by retrograde injection like the vasal artery, and its communication with the epididymo-vasal anastomosis can, therefore, only be by means of capillaries. This has been found to be the case by Joranson, Emmel & Pilka (1929), who, however, claimed that there is a well-marked anastomosis between the testicular artery at the inferior pole of the testis and the vasal artery. This has not been found in the testes examined in this investigation.

The testes of the dog and rat are therefore essentially similar in their vascularization in relation to anastomoses. The dog and rat are not the only mammals in which

the cremasteric artery does not appear to enter into functional (i.e. arterial or arteriolar) communication with the testicular and vasal arteries. In fifty separate mammalian species in which the testicular artery has been injected with radiopaque media having particles which do not pass through capillaries, the only two species other than Man in which the cremasteric artery was filled by retrograde injection were members of the Pongidae (the chimpanzee and lar gibbon).

Experiments

Material and methods

Fifty-six mature male albino rats, weighing 109–303 g., were used for this investigation. Those rats whose testes were examined histologically are described in Table 1. It can be seen from this table that well-circumscribed operations were performed on the animals. In one group a segment of $\frac{1}{2}$ in. of both testicular artery and vein was removed between ligatures in the abdomen. In another group $\frac{1}{2}$ in. of the abdominal course of the testicular artery only was removed. In a third group a $\frac{1}{4}$ in. segment of the testicular artery, together with the veins of the pampiniform plexus, was removed distal to the point at which the testicular artery gives off its epididymal branches, taking care in the operation to avoid any damage to the branch to the cauda epididymidis. In a fourth group the testicular artery only was ligated with fine nylon thread either at a point on the posterior border of the testis or on the anterior border of the testis just after the artery has passed around the inferior pole; in these situations the artery is running on the deep aspect of the tunica albuginea free from veins, since the testicular veins congregate to a point on the postero-superior aspect of the testis to form the pampiniform plexus. A series of five rats was subjected to interruption of the testicular veins only in the abdomen, and in three rats the artery and vein of the vas were interrupted, carefully avoiding damage to the vas itself. Since Joranson, *et al.* (1929) hypothesized, from injection experiments, that increased intratesticular tension, due to the unyielding tunica albuginea, may be an important factor in the production of testicular atrophy following ligation of the testicular vessels, in two further rats the artery and vein were interrupted in the abdomen, while at the same time the tunica albuginea was incised bilaterally.

In all cases the operation on the testicular vessels was performed unilaterally in order to leave the contralateral testis for histological examination as a control. Those animals not described in Table 1, and not examined histologically, were used for investigating the effectiveness of the interruption of the vessels, and any possible post-operative vascular changes, by arteriographic methods.

Results

The results obtained from these experiments are displayed in Table 1. It only remains to summarize and correlate the changes described. On the whole, the cells of the interstitium seem to be more sensitive to vascular changes than the germ cells; in many cases the interstitial cells are degenerated while the tubules contain a few normal germ cells (e.g. rats nos. 723 and 724). The more mature germ cells also seem to be more sensitive to vascular changes than the spermatogonia and Sertoli cells, as described by Schinz & Slotopolsky (1924).

The histological changes following interruption in the abdomen of the testicular artery and vein, or the testicular artery only, occur much less rapidly than after interruption of the artery and vein beyond the anastomosis, or interruption of the artery on the testis. This is reflected in the size of the testis (Table 1, and Pl. 1, figs. 3, 4) and also by the weight of the organ. Thus in rat no. 700 (body weight at death 149 g.), in which the artery and vein were interrupted in the abdomen, the weight of the operated testis after 5 weeks was 475 mg. (contralateral testis 1065 mg.), while in rat no. 682 (body weight at death 150 g.), in which the artery and vein were interrupted beyond the anastomosis of the testicular with the vasal artery, the weight of the testis was only 302 mg. (contralateral testis 1035 mg.). Similarly, in rats nos. 701 and 681, in which the body weight at death of the former was less than that of the latter (173 as against 221 g.) the weight of the operated testis in rat no. 701 was 415 mg. (contralateral testis 1365 mg.), and that in rat no. 681, 386 mg. (contralateral testis 1434 mg.), while in rat no. 690 (body weight at death 217 g.), in which the artery was ligated on the testis, the weight of the operated testis was 390 mg. (contralateral testis 1363 mg.). It should be noted in this connexion that the testis of a rat in which the testicular artery and vein are ligated in the abdomen may undergo a preliminary increase in size as the result of vascular congestion (rats nos. 721 and 722, Table 1); this does not occur when the vessels are interrupted beyond the anastomosis with the vasal artery (rats nos. 702, 703, 723 and 724, Table 1). The histological picture in the operated testes of rats after the same post-operative time interval, shows that, while in rats nos. 681, 682 and 690 there was complete degeneration of interstitial tissue and all germ cells in the seminiferous tubules, in rats nos. 700 and 701 there was still some interstitial tissue present, although showing fibrosis, and in rat no. 700 there were even some tubules containing germ cells up to secondary spermatocytes.

These differences in speed of degeneration are also obvious at later time periods. While, at 7 weeks after operation, the operated testes in rats nos. 102 and 104, in which the artery was ligated on the testis, showed complete replacement by fibrous tissue, in the operated testis of rat no. 706, in which the artery and vein were ligated in the abdomen 7 weeks previously, there were some normal seminiferous tubules. This last testis is actually the only one in the present investigation which displayed normal seminiferous tubules at any time longer than 4 days after interruption of the testicular artery at any point in its course, and it is important to consider whether these normal tubules have regenerated or persisted unchanged. Five weeks after abdominal interruption of the testicular vessels the testis is markedly degenerated; either the tubules are filled with a mass of eosinophilic substance (rat no. 701) or, although containing germ cell elements (rat no. 700), these elements appear degenerate and have been desquamated from the walls of the tubules. It is difficult to conceive of such tubules regenerating a normal structure, and it appears more likely that the normal seminiferous tubules in rat no. 706 have persisted unchanged, or have regenerated after only a partial or minor degree of degeneration. These observations are supported by the fact that in rats nos. 700 and 705 the tubules showing the least degenerative changes are those situated along the epididymal border of the testis, and that the testicular artery, on becoming intratesticular, passes through the length of the testis towards its inferior pole near the epididymal border of the testis

Table 1. *Summary of the animals used and results obtained from division of the testicular vessels*

Rat no.	Operation	Post-operative time interval	Body weight at death (g.)	Size of operated testis (cm.)	Size of control testis (cm.)	Histology
721	Artery and vein in abdomen	48 hr.	237	2·0 × 1·3 × 1·2	2·0 × 1·2 × 1·1	Vascular congestion in interstjtium; dilatation of all vessels, particularly of capillaries in interstitium. Most tubules normal except a few at periphery of testis, which show beginning desquamation of cells in tubules, degeneration of cells and appearance of polynuclear giant cells
722		48 hr.	208	2·3 × 1·4 × 1·2	1·9 × 1·2 × 1·1	Same as rat no. 721, but all tubules show same changes as those at periphery in no. 721
704		4 days	111	1·3 × 0·8 × 0·7	1·4 × 0·9 × 0·8	Interstitium shows degeneration. All tubules degenerated. No germ cells beyond secondary spermatocytes. Some tubules only to primary spermatocytes, the cells themselves being degenerate, with eosinophilic cytoplasm
705		4 days	143	1·4 × 0·8 × 0·7	1·6 × 0·9 × 0·9	Infiltration of interstitium with macrophages and leucocytes. No germ cells beyond degenerate primary and secondary spermatocytes. Infiltration of interstitium less marked along epididymal border of testis
667		4 weeks	264	1·4 × 0·9 × 0·8	2·0 × 1·1 × 1·1	Fibrosis of interstitium. Those tubules at periphery of testis merely filled with a mass of eosinophilic substance. The central tubules show only spermatogonia and Sertoli cells
700		5 weeks	149	1·4 × 0·9 × 0·8	1·8 × 1·1 × 1·0	Interstitium shows degeneration and fibrosis. Tubules show varying degrees of degeneration; some with spermatogonia and Sertoli cells only, others up to secondary spermatocytes, but all showing desquamation of cells from tubular walls
701		5 weeks	173	1·4 × 0·8 × 0·6	1·9 × 1·1 × 1·1	Fibrosis of interstitium. All tubules filled with a mass of eosinophilic substance. No cellular elements visible at all within the tubules
706		7 weeks	156	1·2 × 0·7 × 0·6	1·6 × 1·0 × 0·9	Majority of testis filled with fibrous tissue, leucocytes and macrophages. A few scattered tubules remain in the centre of the testis, all of which have a thickened membrana propria and show varying degrees of degeneration; some are packed with macrophages and polynuclear giant cells, some with a mass of granular basophilic material, with or without giant cells, and others with eosinophilic substance. Along epididymal border of testis are a few perfectly normal seminiferous tubules showing all stages of spermatogenesis up to and including spermatozoa
707		7 weeks	161	0·7 × 0·4 × 0·4	1·6 × 0·9 × 0·9	The only remaining tubules are a few at centre of testis which are profoundly degenerate containing either eosinophilic or basophilic material, with or without polynuclear giant cells, and a few at the periphery mainly containing giant cells. Remainder of testis filled with fibrous tissue containing a few macrophages and leucocytes
708		7 weeks	191	0·8 × 0·5 × 0·4	1·9 × 1·1 × 1·1	
676	Artery only in abdomen	1 week	157	1·4 × 0·9 × 0·8	1·8 × 1·0 × 0·9	Degeneration of interstitium. Most tubules just filled with

giant cells, and a few at the periphery mainly containing giant cells. Remainder of testis filled with fibrous tissue containing a few macrophages and leucocytes

676	Artery only in abdomen	1 week	157	1·4 × 0·9 × 0·8	1·8 × 1·0 × 0·9	Degeneration of interstitium. Most tubules just filled with eosinophilic substance. A few tubules at periphery of testis show spermatogonia, primary spermatocytes and Sertoli cells being desquamated from tubular walls
671		2 weeks	202	1·4 × 0·8 × 0·8	1·9 × 1·0 × 1·0	
675		4 weeks	211	1·6 × 0·8 × 0·8	1·8 × 1·1 × 1·1	Interstitium shows degeneration and fibrosis. All tubules show degeneration; some are just filled with eosinophilic substance, others showing spermatogonia, primary spermatocytes, and Sertoli cells being desquamated from tubular walls
702	Artery and vein after anastomosis with vasal artery	48 hr.	123	1·3 × 0·8 × 0·7	1·4 × 0·9 × 0·8	Interstitium degenerate. Tubules contain degenerate spermatogonia, primary and secondary spermatocytes and Sertoli cells
703		48 hr.	129	1·3 × 0·8 × 0·8	1·5 × 0·9 × 0·9	
681		5 weeks	221	1·2 × 0·8 × 0·7	2·0 × 1·2 × 1·1	Degeneration of interstitium so that tubules in contact with one another. Complete degeneration of all cellular elements in tubules so that tubules merely filled with eosinophilic substance. No adhesions of scrotum to tunica albuginea
682		5 weeks	150	1·1 × 0·7 × 0·6	2·0 × 1·0 × 1·0	
686		8 weeks	234	1·0 × 0·7 × 0·6	2·0 × 1·1 × 1·0	As rats nos. 681 and 682, but membrana propria breaking down in places so that tubular content running from one tubule to another. No adhesions of scrotum to tunica albuginea
723*	Artery only on testis	48 hr.	230	1·6 × 1·0 × 0·9	1·7 × 1·1 × 1·0	Interstitium degenerate. No germ cells beyond secondary spermatocytes in tubules, in most tubules only primary spermatocytes
724*		48 hr.	268	1·6 × 1·0 × 0·9	1·9 × 1·1 × 1·0	
688†		3 weeks	144	1·2 × 0·8 × 0·7	1·8 × 1·1 × 1·1	Interstitium degenerate. All tubules merely filled with eosinophilic substance
450*		4 weeks	295	1·2 × 0·8 × 0·8	2·1 × 1·2 × 1·2	Testis filled with leucocytes and macrophages. Pronounced adhesions all over surface of testis to scrotum and epididymis with ingrowth of capillaries
449*		4 weeks	350	1·3 × 0·8 × 0·7	2·2 × 1·2 × 1·2	Testis just a mass of scar tissue. Pronounced adhesions all over surface of testis
690†		5 weeks	217	1·2 × 0·8 × 0·7	2·0 × 1·2 × 1·1	As rat no. 686. Very slight adhesions at surface of testis
102†		7 weeks	260	0·8 × 0·4 × 0·4	2·0 × 1·3 × 1·1	Testis just a mass of fibrous tissue. Pronounced adhesions all over surface of testis to scrotum and epididymis
104*		7 weeks	276	0·7 × 0·5 × 0·4	2·1 × 1·3 × 1·2	
725	Artery and vein in abdomen with incision of albuginea	4 days	164	1·2 × 0·7 × 0·7	2·0 × 1·2 × 1·1	Interstitium normal and lying in spaces formed by separation of the tubules. All tubules degenerate, many containing only basophilic or granular eosinophilic material, the others showing no germ cells beyond primary spermatocytes. Marked fibrosis around the lesions in the tunica albuginea
726		1 week	184	1·3 × 0·8 × 0·6	1·8 × 1·0 × 1·0	Interstitium shows pronounced fibrosis. Some tubules filled with eosinophilic substance; others show spermatogonia and Sertoli cells, a few also with primary spermatocytes. Many tubules have escaped through the lesions in the albuginea and show considerable proliferation of fibrous tissue with marked adhesions to scrotum and epididymis

* On anterior border of testis. † On posterior border of testis.

(Pl. 1, fig. 1). After interruption of the testicular artery and vein in the abdomen, the blood reaching the testis through the vasal artery can only do so by way of the testicular artery. Only those seminiferous tubules in the immediate neighbourhood of the intratesticular part of the artery are therefore likely to receive nourishment if the blood supply to the testis through the vasal artery, by way of the terminal pathway of the testicular artery, is less efficient than the normal supply. There is some evidence that this is the case, since only one testis in eight after a post-operative period of 4 days or more following interruption of the testicular vessels in the abdomen shows any normal seminiferous tubules.

The above findings indicate that the vasal artery in the rat is of some importance in the blood supply of the testis, since the pathological changes following interruption of the testicular artery are less severe when the vasal artery is still able to contribute blood to the testis. The testicular artery, after giving off its epididymal branches, is an end-artery, and its interruption in this situation is followed by rapid degenerative changes. Ligature of the artery on the anterior or posterior border of the testis seems to make little difference in the speed and ultimate outcome of the degenerative changes, as shown particularly by rats nos. 102 and 104. This is not surprising in view of the fact that the cremasteric artery is only connected with the vasal artery by way of capillaries (as shown by micro-arteriography after the injection of thorotrast into the thoracic aorta of six rats) across the ligamentum inguinale,* and that no evidence has been found, in the rat, of any vessel larger than capillary size traversing the ligamentum testis.*

There are two predominant histological pictures in the seminiferous tubules following interruption of the testicular artery. In one the nuclei are seen to undergo pyknosis or karyolysis and to disappear eventually leaving the seminiferous tubules filled with a mass of eosinophilic substance, i.e. a process of hyaline degeneration (Pl. 1, fig. 5). In the other the picture is one of degeneration with replacement fibrosis. These two processes seem to have no definite relation to the operative procedure. Thus replacement fibrosis is the end result of ligature of the artery on the testis in rats nos. 102 and 104, but it also occurs in rat no. 706 (Pl. 2, fig. 1) in which the artery and vein were interrupted in the abdomen.

Adhesions of the tunica albuginea to scrotum and epididymis are more common in the cases in which the artery is ligated on the testis. In these cases the testis was necessarily handled in the operative procedure, this being unnecessary in other operations, except in the animals in which the tunica albuginea was incised. Whenever adhesions are found attaching scrotum to tunica albuginea there is an ingrowth of capillaries into the testis, as evidenced by the blueing of the testis following injection of methylene blue solution into the aorta. This was demonstrated in experimental animals in which adhesions occurred after ligation of the artery on the testis, and the ligamentum testis remained unstained in such cases.

The course of the degenerative changes following interruption of the testicular artery only in the abdomen is similar to that in animals in which the testicular artery and vein were interrupted. There is no evidence, therefore, that in the rat

* These terms being used in the sense described by Weber (1927), the ligamentum inguinale being that part of the gubernaculum testis between the cremaster sac and cauda epididymidis, and the ligamentum testis that part between the cauda epididymidis and inferior pole of the testis.

spermatogenesis is more likely to continue after interruption of the artery alone than after interruption of the artery and vein, as claimed for the dog by Wangensteen (1927). This may be explained by the fact that, in the rat, only a proportion of the venous blood from the testis is returned through the testicular veins; a considerable fraction passes directly into the iliac vein by way of a short, wide channel. This venous connexion may also explain why, in the five rats in which the testicular veins only were interrupted in the abdomen, there is no diminution in size of the affected testes, and no histological changes up to 4 weeks after operation. Unfortunately, it is difficult to interrupt all venous drainage from the testis in the rat, since it is almost impossible to section all the veins of the pampiniform plexus without damaging also the testicular artery. Section of the vasal vessels alone, up to 4 weeks after operation, produces no changes in the testis. Bilateral incision of the tunica albuginea does not appear to exert any favourable influence on the course of the changes following interruption of the testicular vessels, as shown by rats nos. 725 and 726.

In all cases the tubules of the contralateral testes show histological evidence of perfectly normal spermatogenesis. Examination by arteriography of the vascularization of the testes after the operations used in this investigation displays the complete effectiveness of the operative procedures. Although there is some dilatation of the vasal artery after the interruption of the blood flow through the testicular artery, the degree of this dilatation is variable. Whereas, in some cases the diameter of the vasal artery (as measured on radiographs) is little greater than normal (one-half the diameter of the testicular artery), in other cases the diameter became equal to that of the testicular artery. The degree of dilatation tends to be greater in those cases in which the testicular artery and vein have been interrupted in the abdomen.

THE ANASTOMOSIS OF THE CREMASTERIC AND VASAL ARTERIES WITH THE TESTICULAR ARTERY IN THE HUMAN TESTIS

Practically all the previous work on the effect of ligature of the testicular vessels in experimental animals was performed on dogs, since it was assumed that the blood supply of the testis and epididymis in this animal is similar to that in Man, and that the results obtained from these experiments are therefore applicable to the human testis (see Neuhof & Mencher, 1940). In fact, however, there are marked differences in the manner of anastomosis of the testicular artery with other vessels, as will be apparent from the following account.

Most workers who have made observations on the testicular artery in Man have commented on the cremasteric and vasal arteries (see Harrison & Barclay, 1948, for references), but it was not until the observations of Picqué & Worms (1909) that the extent and variability of the anastomosis of these vessels with the testicular artery were realized. The investigations of these two workers followed the claim by Mignon (1902) (also, however, made by Bevan, 1903), that, in order to ensure placement of an undescended testis into the scrotum, section of all the vessels in the spermatic cord except those accompanying the vas is often necessary. As a result of their observations, Picqué & Worms (1909) described the only communication of the vasal artery with the testicular artery as occurring by way of the epididymal branch of the latter; the cremasteric artery was found to anastomose with the other two vessels in all of twenty-four specimens examined, in four cases directly with the testicular

artery, and in the remainder with the loop formed by the epididymo-vasal anastomosis. No indication was, however, given of the functional importance of these anastomoses. An attempt has therefore been made by arteriographic methods to examine the anastomoses of the cremasteric and vasal arteries with the testicular artery, and the possible functional value of these anastomoses.

Material and methods

Twenty-four fresh post-mortem human testes from thirteen individuals were used for the investigation. The ages of the subjects from which these specimens were obtained ranged from 21 to 75 years. The vasal artery was injected in all cases with 'Chlorbismol' in 15 % suspension at a pressure of about 100 mm. of mercury, in order that the results obtained may be directly comparable. Radiography was done as in the experiments on the rat testis. Dissection was also used on the injected specimens, and diameters of vessels were measured to the nearest 0·1 mm. on the radiographs by taking the average of the values at ten points selected at random along the course of the vessels. As in the experiments on the rat testis, 'Chlorbismol' will only display functional channels of greater than capillary size.

Results

It was soon realized that there are variations in the manner of anastomosis of the three vessels supplying the testis. In every case the type of anastomosis already described in the dog and rat, namely an anastomosis of the vasal artery with the testicular artery by way of the epididymal branch of the latter, is present. But in all of the testes an additional anastomosis is also seen, in which the vasal artery anastomoses with the testicular artery directly at the inferior pole of the testis, without the intermediation of any epididymal branch of the testicular artery. The manner of this anastomosis varies. In fifteen out of the twenty-four testes the anastomosis takes place by means of a large vessel (about 0·5 mm. in diameter) which anastomoses directly with one of the major branches of the testicular artery by passing across from the cauda epididymidis to the inferior pole of the testis (Pl. 2, fig. 2). In the other nine testes the anastomosis is effected by several smaller vessels in this situation which pass into the testis to anastomose with some of the finer intra-testicular branches of the testicular artery (Pl. 2, fig. 3), or the testicular artery itself, as it is lying on the deep aspect of the tunica albuginea at the inferior pole of the testis (Pl. 2, fig. 4). In this respect the vascularization of the human testis differs from that of the testes of the dog and rat, which possess no large vascular channels connecting cauda epididymidis with the inferior pole of the testis.

Owing to the two types of anastomosis just described between the vasal and testicular arteries, injection of the former gives rise to retrograde filling of the latter.

However, filling of the cremasteric artery after injection of the vasal occurred in only seventeen out of the twenty-four testes. The anastomoses permitting this retrograde injection of the cremasteric artery, are very complex when present (Pl. 2, figs. 2, 3). In all cases the cremasteric artery anastomoses directly or indirectly with the epididymo-vasal system of vessels, in six cases this being effected by the cremasteric artery anastomosing primarily with the vessels of the caput epididymidis which then join with the epididymo-vasal system at the cauda

epididymidis. This latter anastomosis was effected in four out of the six cases by a slightly tortuous vessel running along the body of the epididymis and seeming to be in direct continuity with the cremasteric artery.

The diameter* of the testicular artery, as measured on the radiographs, varies but little, the smallest diameter observed being 0·7 mm. and the largest 1·1 mm. (average 0·9 mm.). The diameters of the vasal and cremasteric arteries, however, vary within wide limits. Thus the smallest diameter of the vasal and cremasteric arteries is 0·1 mm., the largest diameters being 1·0 and 0·7 mm. respectively. The vasal artery may be a mere thread-like vessel (Pl. 2, fig. 4) or a vessel of proportionately large calibre (Pl. 2, fig. 3), while, as has already been noted, the cremasteric artery may fail to enter into functional anastomosis with the other two vessels. It is also interesting to note that the sum of the diameters of the vasal and cremasteric arteries is at least equal to the diameter of the testicular artery in eight out of twenty-four cases in this investigation, while in two cases the vasal artery alone has a diameter equal to that of the testicular artery.

DISCUSSION

There is no unanimity in the literature concerning the effects of division of the testicular vessels in Man. Mignon (1902) advocated the section of all blood-vessels in the spermatic cord, except those accompanying the vas, in order to obtain fixation of the testis in the scrotum in the operation of orchiopexy, asserting that in undescended testes the vas is no shorter than normal but the vessels are always very small and form a shortened and inextensible cord. Lucas-Championnière (1902), and Sebileau (1902) claimed, however, that the three cases which Mignon had presented to demonstrate this operation all showed atrophy of the testis. Colles (quoted by Moty, 1902) had previously described the anastomosis between testicular, vasal and cremasteric arteries as being absent in 7 % of cases. Bevan (1903) concluded that all vessels in the cord, except those accompanying the vas, could be safely sacrificed in operations for varicocoele. Corner & Nitch (1906), in a follow-up of 100 cases of high operation for varicocoele in which a 2 in. segment of the pampiniform plexus is removed, observed changes in the consistency of the testis suggesting fibrosis in 90 % of cases. No histological observations were, however, made in this investigation. Wells (1948) has pointed out that the testicular artery lies in close relationship to the veins and is likely to be injured if the operation for varicocoele is done carelessly; he states that the results of Corner & Nitch (1906) are probably due to the inclusion of the testicular artery in the ligatures, that the operation as commonly described is carried out in such a way as to sacrifice the testicular artery deliberately in the mass division of the pampiniform plexus, and that subsequent atrophy of the testis is no cause for wonder.

Mixter (1924), in describing his operation for undescended testis in children, claims that in 10 % of all cases (particularly in the intra-abdominal varieties of undescended testis) the testicular vessels must be sectioned; atrophy of the testis then occurs in 85 % of cases. Atrophy may also ensue when the testicular vessels

* In order to diminish distortion of vessel diameter on the radiograph to negligible proportions, a tube filament—film distance of 24 in., was chosen. Since the important measurements here are relative, i.e. the comparison of the diameters of the vessels, the degree of distortion, being constant, is not important.

are not divided (12 % of cases), when it is probably due to accidental injury to the vessels at operation.

Burdick & Higinbotham (1935) have divided the spermatic cord in 200 cases as an aid to operation on selected types of inguinal hernia. A ligature is placed about the cord at its emergence through the internal ring and a second one as it enters the scrotum, the intervening tissue being excised. It is not specifically stated whether the vasal artery is included in the ligature, and it must only be presumed that the cremasteric artery is left intact. They record clinical atrophy of the testis in only twenty-two out of seventy-nine cases, although a fairly common observation was post-operative swelling of the testis. These results may be criticized on the grounds that no observations were made on the state of spermatogenesis in the operated testes by semen analysis or histological examination by testicular biopsy. Testes recorded as being 'normal in size' need not necessarily display active spermatogenesis, and it may be difficult from clinical examination alone to determine whether the seminiferous tubules are normal. It is significant that all cases in which the cord was divided bilaterally showed normal-sized testes on both sides. Neuhof & Mencher (1940) similarly used complete severance of the spermatic cord (including severance of the vas deferens and its artery) to achieve effective hernioplastic closure in selected cases; the operation is performed in the inguinal canal. They found clinical atrophy of the testis in eight out of nineteen cases. In one case the operated testis was examined histologically, and although 'all stages of spermatogenesis' were evident in some of the seminiferous tubules, they admit of some abnormality in the transformation of spermatids into spermatozoa, and they describe only a few tubules of the epididymis as containing spermatozoa, which had rounded heads and no tails. In absence of further, more definite, observations by semen analysis or testicular biopsy it is therefore difficult to accept unequivocally their conclusion that the human testis is only insignificantly altered following complete division of the cord.

There is, therefore, some lack of agreement as to the effect of section of the testicular vessels. On the one hand, authorities such as Bailey (1936) claim that 'once the spermatic vessels are damaged seriously, atrophy of the body of the testis follows as surely as night follows day', while on the other, some surgeons (Neuhof & Mencher, 1940) claim atrophy in no more than 50 % of cases after complete severance of the cord. On the results of the present investigation several possible explanations of these discrepancies can be made.

Most observers are in agreement that when the testicular vessels supplying a mal-descended testis are damaged atrophy of the testis ensues. But the vessels supplying such a testis are reported as being far from normal; several investigators (e.g. Mignon, 1902) have recorded them as being very small, while Staemmler (1923) has described a hypoplasia of the testicular artery supplying the undescended testis, with intimal proliferation and hyaline degeneration of the walls of its smaller branches, a condition which he believes (probably erroneously) to be primarily responsible for the testicular maldevelopment. Since most operations for undescended testis are carried out on young individuals, another factor to be taken into account is the comparative immaturity and poorer anastomotic connexions of the testicular circulation. This was demonstrated by injection of the testicular artery with 'Chlorbismol' in the testes of two boys, aged 8 and 10 years. In the former only the vasal artery

was filled by retrograde injection through two small anastomotic channels on the body of the epididymis. In the latter neither the vasal nor cremasteric arteries was filled, the epididymis being profusely vascularized by the testicular artery. It can therefore be conceded that any damage to the testicular vessels during orchiopexy may lead to atrophic changes in the majority of cases, particularly since the testis has been in an adverse environment (see Moore, 1926) before operation. The results accruing from section of the testicular artery in such cases consequently should not be applied to herniorrhaphy and varicocoele operations in adults, since the testicular circulation in healthy adult men is composed of an efficient arterial anastomotic system in about one-third of all cases. In such cases, in any operation in which only the testicular artery is damaged there is a likelihood that the cremasteric and vasal arteries could take over its function in the presence of an efficient venous drainage. If, however, both the testicular and vasal arteries are sectioned, the only remaining arterial supply to the testis is through the cremasteric artery, since no case has been found in the present investigation in which there is an arterial or arteriolar connexion between the testicular artery and the pudendal vessels. The cremasteric artery in Man is a most variable vessel (functionally probably less efficient than the vasal artery in the rat), as shown, for example, by the complete absence of important anastomotic connexion with the testicular and vasal arteries in about one-third of cases, and it is therefore difficult to envisage this artery alone maintaining an adequate testicular circulation. It also follows that, in the radical operation for varicocoele, there will be a greater possibility of survival of the testis if the operation is performed in the inguinal canal than if it is carried out below the external ring; in the case of inadvertent damage to arteries (always providing that the vas and its vessels are avoided) the danger of interruption of the testicular artery would be greater below the external ring. In this position the testicular veins have a more complicated arrangement than in the inguinal canal; the nearer the testis the more voluminous the pampiniform plexus, and since the testicular artery is always ensheathed by the veins of pampiniform plexus there is greater difficulty in isolating this vessel near the testis. The venous drainage, although of less importance due to the greater profusion of anastomotic connexions, must also be considered. Javert & Clark (1944) maintain that the optimum point of ligation of the testicular veins in operations for varicocoele is at the internal abdominal ring, which, while eliminating the static column of blood does not impair the collateral circulation through the cremasteric and vasal veins. The venous circulation through alternative channels to the testicular veins has already been noted as of no small importance in the rat.

Finally, it is not valid to apply unconditionally the results of animal experiments to the problems resulting from ligation of the testicular vessels in Man. Animal experiments can only give some indication of the pathological processes involved. It has been seen that the vasal artery which, in the rat, is only one-half the diameter of the testicular artery, can play a small part in the delay (and occasionally partial circumvention) of degenerative processes. In Man, however, the vasal artery is much larger, proportionately, than its counterpart in the rat, has more efficient anastomotic connexions with the testicular artery, and has the assistance of the cremasteric artery in some cases in the nourishment of the testis. The results of division of the testicular artery must therefore be very different in Man and other mammals. It

has also been noted (Harrison & Barclay, 1948) that the testicular artery in Man (at least in the European) differs from that in the majority of mammals by being almost straight in its course to the testis.

The results of incision of the albuginea when the testicular vessels are interrupted do not seem to be encouraging in the rat. The claim by Baker & Evoy (1942) that 'decompression' of the human testis, by incising the albuginea in one case of painless enlargement following herniorrhaphy, produced 'no discernible abnormality of the involved testicle', in contrast to a similar, untreated case, must therefore await histological confirmation.

Once the testicular artery or its branches have passed into the testis, on the deep aspect of the tunica albuginea, such vessels are functionally end-arteries after the anastomosis at the inferior pole of the testis. Consequently, the conclusion (Harrison & Barclay, 1948) that great care must be exercised to avoid ligaturing the testicular artery in its superficial position under the tunica albuginea in the surgical treatment of cryptorchids, still obtains.

SUMMARY

1. The degenerative processes resulting from interruption of the testicular artery at various points in relation to its anastomotic connexions have been examined in the rat. The results indicate that the vasal artery in the rat is of some importance for the blood supply of the testis, since the degenerative changes following interruption of the testicular artery are less severe when this artery is still able to contribute blood to the testis.

2. The vascularization of the testis in the dog and rat differs from that in Man in that a direct anastomosis of the testicular artery with the vasal and cremasteric arteries is absent. Unconditional application of the results of division of the testicular vessels in experimental animals to Man is therefore not valid.

3. Examination by arteriographic methods of the vascularization of the human testis demonstrates that the sum of the diameters of the cremasteric and vasal arteries is at least equal to the diameter of the testicular artery in one-third of all cases examined. In approximately one-third of cases the cremasteric artery does not enter into functional anastomotic connexion with the testicular and vasal arteries.

4. The importance of these observations for the results of division of the testicular artery in Man is discussed, and several possible explanations of the discrepancies in the literature concerning the effects of ligature of the testicular artery in Man are proposed.

I am indebted to the late Dr A. E. Barclay, and Dr G. S. Dawes for permitting the use of the micro-radiography apparatus at the Nuffield Institute for Medical Research. I also wish to thank Mr L. G. Cooper for his technical assistance. May and Baker Ltd., kindly supplied all the 'Chlorbismol' used.

REFERENCES

BAILEY, HAMILTON (1936). *Diseases of the Testicle.* London: H. K. Lewis.

BAKER, J. W. & EVOY, M. M. (1942). Insult to the testicle in herniorrhaphy. *Surg. Gynec. Obstet.* **75**, 285–288.

BARCLAY, A. E. (1947). Micro-arteriography. *Brit. J. Radiol.* **20**, 394–404.

BEVAN, A. D. (1903). The surgical treatment of the undescended testicle. A further contribution. *J. Amer. med. Ass.* **41**, 718–724.

BURDICK, C. G. & HIGINBOTHAM, N. L. (1935). Division of the spermatic cord as an aid in operating on selected types of inguinal hernia. *Ann. Surg.* **102**, 863–874.

CAPURRO, M. A. (1902). Sulla circolazione sanguigna normale e di compenso del testiculo. *Anat. Anz.* **20**, 577–598.

COOPER, ASTLEY (1830). 'On the Anatomy of the Testis.' *Observations on the Structure and Diseases of the Testis*, Pt. 1, 1–55. London: Longman, Rees, Orme, Brown and Green.

CORNER, E. M. & NITCH, C. A. R. (1906). The immediate and remote results of the high operation for varicocele. *Brit. med. J.* **1**, 191–193.

ENDERLEN, H. (1896). Klinische und experimentelle Studien zur Frage der Torsion des Hodens. *Dtsch. Z. Chir.* **43**, 177–186.

GRIFFITHS, J. (1896). The effects upon the testes of ligature of the spermatic artery, spermatic veins, and of both artery and veins. *J. Anat., Lond.*, **30**, 81–105.

HARRISON, R. G. & BARCLAY, A. E. (1948). The distribution of the testicular artery (internal spermatic artery) to the human testis. *Brit. J. Urol.* **20**, 57–66.

IWASITA, K. (1939). *Jap. J. Derm. Urol.* **45**, 126–135. (Quoted by Baker & Evoy, 1942.)

JAVERT, C. T. & CLARK, R. L. (1944). A combined operation for varicocoele and inguinal hernia. *Surg. Gynec. Obstet.* **79**, 644–650.

JORANSON, Y., EMMEL, V. E. & PILKA, H. J. (1929). Factors controlling the arterial supply of the testis under experimental conditions. *Anat. Rec.* **41**, 157–176.

LUCAS-CHAMPIONNIÈRE, M. (1902). Discussion on Mignon's (1902) paper. *Bull. Soc. Chirurgie Paris*, **28**, 756–758.

MIFLET, J. (1879). Ueber die pathologischen veränderungen des Hodens, welche durch Störungen der localen Blutcirculation veranlasst werden. *Arch. klin. Chir.* **24**, 399–428.

MIGNON, A. (1902). Traitement de l'ectopie testiculaire par la section de tous les vaisseaux du cordon spermatique. *Bull. Soc. Chirurgie Paris*, **28**, 752–756.

MIXTER, C. G. (1924). Undescended testicle. Operative treatment and end-results. *Surg. Gynec. Obstet.* **39**, 275–282.

MOORE, C. R. (1926). The biology of the mammalian testis and scrotum. *Quart. Rev. Biol.* **1**, 4–50.

MOTY, M. (1902). Discussion on Mignon's (1902) paper. *Bull. Soc. Chirurgie Paris*, **28**, 780.

NEUHOF, H. & MENCHER, W. H. (1940). The viability of the testis following complete severance of the spermatic cord. *Surgery*, **8**, 672–685.

PICQUÉ, R. & WORMS, G. (1909). Les voies anastomotiques de la circulation artérielle testiculo-épididymaire. *J. Anat., Paris*, **45**, 51–64.

SCHINZ, H. R. & SLOTOPOLSKY, B. (1924). Beiträge zur experimentellen Pathologie des Hodens und zur Histologie und Histogenese des normalen Hodens, der Hodenatrophie und der Hodennekrose. *N. Denkschr. schweiz. naturf. Ges.* **61**, 27–137.

SEBILEAU, P. (1902). Discussion on Mignon's (1902) paper. *Bull. Soc. Chirurgie Paris*, **27**, 759–760.

STAEMMLER, M. (1923). Über Arterienveränderungen im retinierten Hoden. *Virchows Arch.* **245**, 304–321.

WANGENSTEEN, O. H. (1927). The undescended testis. An experimental and clinical study. *Arch. Surg., Chicago*, **14**, 663–731.

WEBER, M. (1927). *Die Säugetiere Einführung in die Anatomie und Systematik der Recenten und Fossilen Mammalia.* Band 1. Jena: Gustav Fischer.

WELLS, C. (1948). The Testicles and Spermatic cord. Chaps. L and LIV in Winsbury-White, H. P. *Textbook of Genito-urinary Surgery*, pp. 535–536, 586–588. Edinburgh: E. and S. Livingstone Ltd.

EXPLANATION OF PLATES

Plate 1

Fig. 1. The arterial supply of the rat testis as visualized by radiography after the injection of 'Chlorbismol' into the thoracic aorta. Natural size. *t.a.*, testicular artery; *v.a.*, vasal artery; *c.a.*, cremasteric artery. The testicular artery convolutes before reaching the testis, and gives off branches to epididymis and fatty body. The branch to cauda epididymidis anastomoses with the vasal artery, and this anastomosis sends off a small branch on to the scrotum into the territory of terminal distribution of the cremasteric artery, not, however, forming a functional anastomosis with it.

Fig. 2. The arterial supply of the dog's testis as visualized by radiography after the injection of 'Chlorbismol' through the testicular artery. Natural size. *t.a.*, testicular artery; *v.a.*, vasal artery. The testicular artery convolutes before reaching the testis, and its branch to the cauda epididymidis anastomoses with the vasal artery. There are no vessels passing from cauda epididymidis to inferior pole of testis.

Fig. 3. The appearance of operated (on left) and unoperated (on right) testes 4 weeks after interruption of the testicular artery and vein in the abdomen. ($\times \frac{4}{5}$.)

Fig. 4. The appearance of operated (on left) and unoperated (on right) testes 4 weeks after ligature of the testicular artery on the posterior border of the testis. Note that the epididymis on the operated side is normal in size. ($\times \frac{4}{5}$.)

Fig. 5. Photomicrograph of the seminiferous tubules in the testis of a rat (682) in which the testicular artery and vein were interrupted after the anastomosis with the vasal artery 5 weeks previously. The interstitial tissue is degenerate, and there is pronounced hyaline degeneration of the contents of the tubules, no germ-cell elements persisting. ($\times 112$.)

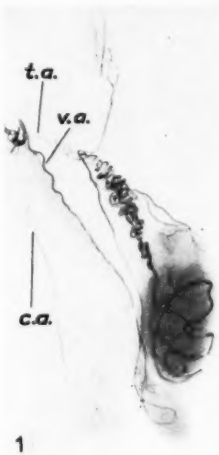
Plate 2

Fig. 1. Photomicrograph of the testis of a rat (706) in which the testicular artery and vein were interrupted in the abdomen 7 weeks previously. The figure shows an area in that part of the testis in which there is considerable fibrosis with some infiltration of leucocytes and macrophages. Only a few degenerate tubules remain; in one of the tubules a polynuclear giant cell may be seen. ($\times 86$.)

Fig. 2. Radiograph of the left testis and spermatic cord from a man aged 71 after the injection of 'Chlorbismol' into the vasal artery (*v.a.*). This artery enters into anastomosis with epididymal branches of the testicular artery (*t.a.*) and also directly with the testicular artery towards the inferior pole of the testis. The cremasteric artery (*c.a.*) is also injected. Natural size.

Fig. 3. Radiograph of the right testis and spermatic cord from a man aged 58 after the injection of 'Chlorbismol' into the vasal artery (*v.a.*). The cremasteric artery (*c.a.*) is clearly seen. The anastomosis formed by the vasal artery and epididymal branches of the testicular artery (*t.a.*) sends off several small vessels into the testis to anastomose with the finer intratesticular branches of the testicular artery. The arrows point to the anastomoses made by the cremasteric artery with the other two vessels. A branch is given off from the cremasteric artery which, at the upper arrow, is seen to send off a side branch to the epididymo-vasal anastomosis, and then pass on to anastomose with the vasal and testicular arteries directly. The terminal branches of the cremasteric artery pass to the cauda epididymidis and here (at the lower arrow) are seen to join with the epididymo-vasal anastomosis. Natural size.

Fig. 4. Radiograph of the left testis and spermatic cord from a man aged 75 after the injection of 'Chlorbismol' into the vasal artery (*v.a.*) which is seen here to have divided into two vessels of smaller diameter. The cremasteric artery (*c.a.*) is a very small vessel. The epididymo-vasal anastomosis forms a complex network of small vessels at the cauda epididymidis, from which a few fine vessels pass across to the inferior pole of the testis to anastomose directly with one of the branches of the testicular artery (*t.a.*). Natural size.

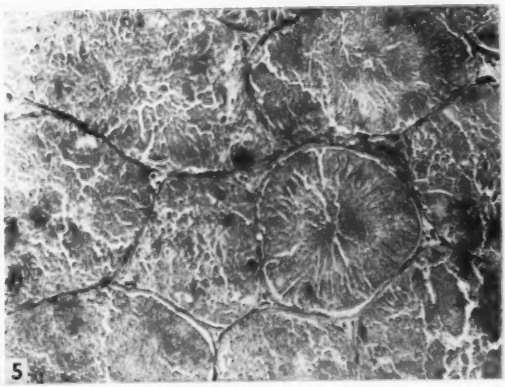


1

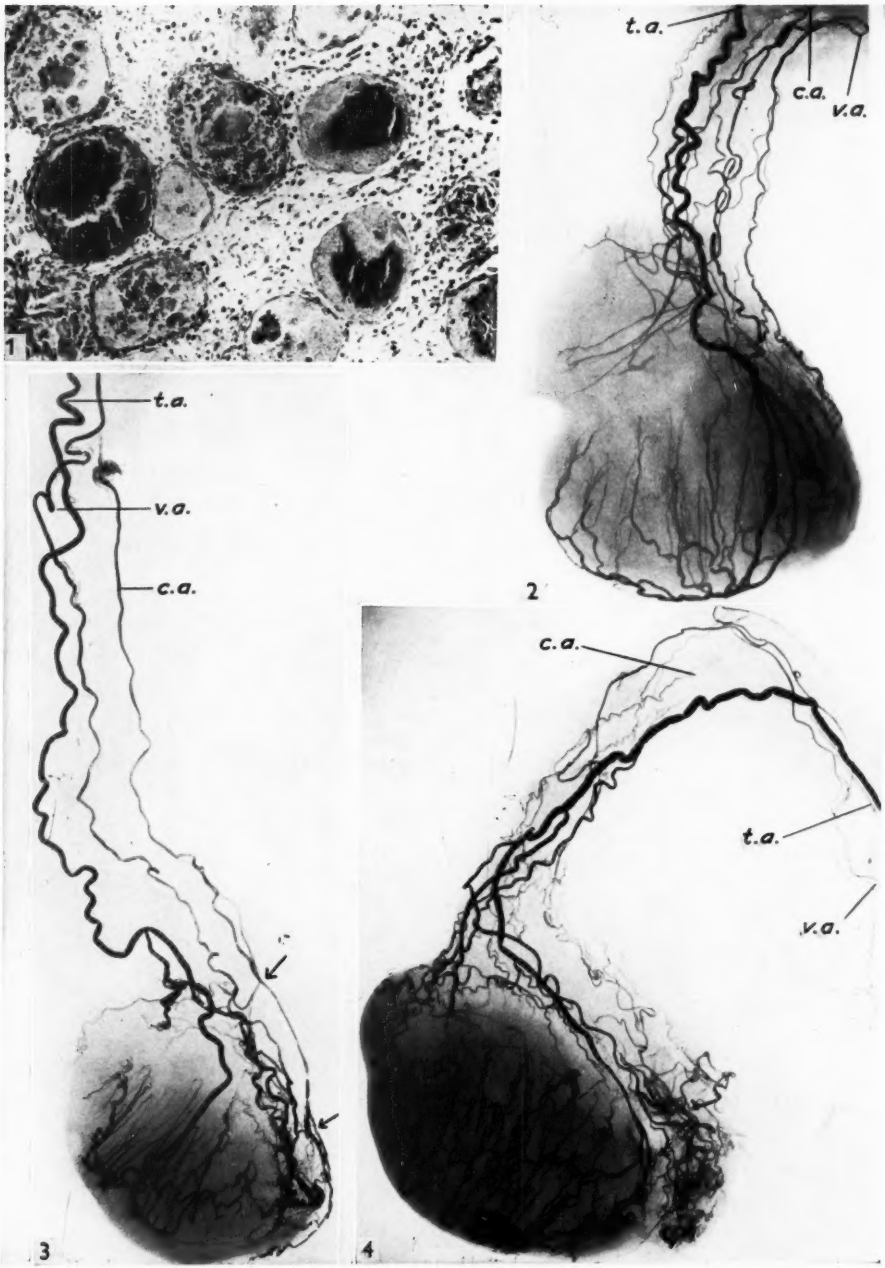
3

4

2



5



HARRISON—THE VASAL AND CREMASTERIC ARTERIES TO THE TESTIS

REVIEWS

The Anatomy of Semnopithecus entellus. By A. Ananthanarayana Ayer. Madras: The Indian Publishing House, Ltd., 1948. (Pp. 182, with 99 figs., including 8 plates.) Price 20*s*.

The mantle of the late U. V. Nayak as primatologist appears to have fallen upon his pupil and successor, the author of this book. The Madras Medical College is to be congratulated on this fact. The present work is the unrevised M.Sc. thesis presented in 1942 and fills an important gap in our knowledge of the comparative anatomy of the Catarrhine monkeys since no monographic study has hitherto been made of any Asiatic member of the important family Colobidae, the only previous comprehensive study being that of Polak on the African genus *Colobus*.

The account is confined to the gross anatomy and deals, in systematic order, with external characters, skeleton, joints, myology, the viscera and neurology. These are not all of equal merit, but serve as a basis for further studies, since previously published data are reviewed and compared with the author's own findings, whilst comparisons are also made with conditions in other Primates. Unfortunately, the work is based upon the dissection of only three individual monkeys, supplemented by one articulated and one disarticulated skeleton. Of these the age of the articulated skeleton alone is stated. Judging from the photographs, some, at least, of the preserved material was immature.

This should not detract, however, from the general value of the study as a work of reference or as an introduction to Primate anatomy. The general format is satisfactory, the paper of good quality and the print clear. This also applies to the text figures, but the plates, printed on rather poor quality art paper, do not do justice to the rest of the book. They could well have been omitted as they carry little weight; the photograph of the male genitalia, for example, is out of focus and quite unnecessary in view of the details given in fig. 77.

The stomach naturally receives a good deal of attention and is well described, though the account is hardly systematic; it gives no indication of the normal relationship of the different parts of the organ to each other when *in situ*, nor are the peritoneal relations well discussed. One or two minor errors are noted. After meticulous care in the introduction regarding the correct identification and nomenclature of his material, the author elsewhere permits inconsistencies to creep in. Thus the rhesus monkey (*Macaca mulatta*) is referred to as *Macacus rhesus*, but the bonnet monkey is sometimes called *Macacus radiatus* and elsewhere *Macaca radiata* or *Macacus sinicus*. But any anatomist who is not also a systematist might be forgiven for confusion, especially when quoting from early authorities. Polak is misquoted throughout as 'Polok'.

The last two chapters consisting of 'summary of significant observations' and 'affinities of *Semnopithecus*' constitute a judicious rounding off of the work and the general conclusion that *Semnopithecus*, in its anatomy, displays a curious blend of primitive and progressive features seems to be well justified.

W. C. Osman Hill

Detailed Atlas of the Head and Neck. By Raymond C. Truex and Carl E. Kelever. (Large 4vo: pp. xiii + 162.) New York: Oxford University Press, 1948. 75*s*. net.

This lavishly illustrated and sumptuously produced atlas comprises 136 figures, in colour, monochrome or a combination of both, displaying the regional anatomy of the head and neck. Of this number, 82 depict regional dissections, 20 show frontal and 18 show horizontal sections, and a series of 20 cerebral figures includes 6 representing the fibre constitution of the hemisphere. The figures are done from original preparations and specimens, specially made or selected for this work.

The value of any anatomical atlas must remain largely a matter of personal prejudice, for probably no two opinions will agree as to optimum scope of content. The present atlas appears uneven in this particular, for some structures of the chosen region receive a treatment more elaborate than that bestowed upon others of equal importance. Thus four figures (Figs. 96–99) are devoted to enlarged reproductions of the auditory ossicles (without their associated ligamentous or vascular apparatus), yet the tympanum itself is accorded a less liberal attention. The thyroid gland receives no particularly detailed treatment, whilst the parathyroids are altogether omitted, and the utility of four cerebellar figures in a work of this nature seems questionable.

Anatomical blemishes appear to be few. We note, however, that in Fig. 6 the ala nasi is not innervated by the maxillary nerve, while the auriculo-temporal nerve supplies overmuch of the skin of the pinna. The jugular lymph trunks and the submental lymph glands are nowhere figured. An obvious socia parotidis is wanting in Fig. 18. The superficial Sylvian vein is depicted (Fig. 31), but the deep Sylvian and the basal veins are ignored elsewhere. In Figs. 24, 25 and 65 the sinus of Morgagni is not labelled. The cervical vertebrae (Figs. 83, 85) are practically devoid of metapophyseal tubercles and their anterior ligamentous areas are too feebly pronounced. The atlas vertebra (Fig. 68) is given a 'lateral process', apparently a new term for the posterior bar of the transverse process. The upper limit of origin of M. temporalis in Fig. 91 is not coincident with the inferior temporal line. The picture of the basis cranii interna (Fig. 94) shows the petrous to be devoid of its customary vascular imprints from the superior and inferior petrosal sinuses: and the sphenoidal lingula is insufficiently emphatic. The external auditory meatus (particularly the tympanic plate) is poorly represented in Figs. 90, 91: these illustrations, together with Figs. 92, 94, demonstrate the general unsuitability of monochrome 'wash' as a medium for depicting osseous structures or tissue. It is unfortunate that in Fig. 104 the several elements bounding the ostium maxillare remain unlabelled and that the dissection of the temporal bone was not carried to a deeper and more informative level.

The standard of artistic execution is high and reflects a commendable fidelity to the original preparations. The restriction, however, of bright colours to neural and vascular structures only is perhaps not altogether happy. For (in the horizontal and frontal sections particularly) these stand out too vividly against a general monochromic background and the eye is deflected by minor branches and twigs from more important structures. Had the muscular, glandular and other masses been appropriately tinted this defect would have been obviated.

The quality of the paper, the printing and the general production are superb, and there is an exhaustive index. Every accurate reproduction of a good dissection or section is of value, and this atlas should therefore prove serviceable to the medical student. We doubt, however, whether the ophthalmologist, the otologist and the laryngologist will find it sufficiently comprehensive for professional reference.

A. J. E. CAVE